

**THE PREVALENCE OF ANTIBODIES TO
BOVINE LEUKEMIA VIRUS, NEOSPORA CANINUM AND RISK
FACTORS, AND BIOSECURITY PRACTICES IN BEEF
COW-CALF HERDS IN CANADA**

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Western College of Veterinary Medicine

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Saskatoon

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ABSTRACT

A total of 4,778 cows from 179 herds were tested for antibodies to *N. caninum* using a commercially available ELISA. *Neospora caninum* herd-level seroprevalence ranged from 25.0% to 75.9% (a herd was considered positive with ≥ 2 cows testing positive). The true cow prevalence was estimated as 5.2% (95% CI= 4.6 – 5.8). “Pre-calving use of dry lots”, “separation of cow-calf pair from other cows after calving”, “use of standing water in summer”, “use of running water in winter”, “feeding heifers with manure handling equipment”, “abortion and stillbirths left for canids” and “number of sightings of wild canids per year” (categorized into three categories: less than 10 times per year, 11– 25 times per year, and greater than 26 times per year) were positively associated with herd serological status.

However, “washing boots between visits to livestock farms” was negatively associated with serological status. These 8 variables were included in a multivariable logistic regression model. Province and herd size were considered potential confounders and kept in the model regardless of significance. Only 4 variables remained significant in the final model. Risk factors associated with prevalence included the use of dry lots/corrals as pre-calving area (OR=2.8; 95% CI=1.3 – 6.2), the use of natural standing water in summer (OR=3.2; 95% CI=1.31 – 8.0), and leaving abortions/stillbirths for dogs or wild canids (OR=2.5; 95% CI=1.0 – 5.9).

As the frequency of sighting coyotes and foxes increased so did herd seroprevalence to *N. caninum*. Risk factors suggested the likely role of horizontal transmission in the transmission of *N. caninum* in these beef cow-calf herds. Beef herd managers might consider biosecurity practices such as preventing the access of wild canids to fetuses and stillbirths thereby preventing pasture contamination and controlling contamination of water source with oocyst of *N. caninum* thereby reducing chances of infection.

A herd was considered positive for Bovine leukemia virus (BLV) if ≥ 1 animal tested positive. Estimates of cow-level seroprevalence was 1.01% (95% CI= 0.73% – 1.29%) while herd seroprevalence was 12.4% (95% CI= 7.57 – 17.23). Potential risk factors examined for BLV transmission included the use of blade or surgical castration without disinfection between animals, using gouger and saw dehorning methods, multi-use of common rectal sleeve between cows without disinfection and the use of communal pasture where mating occurred.

No associations existed between potential risk factors and seropositivity to BLV because the number of herds testing positive to BLV were too few to find any association. However, management practices observed in this study may have the potential to transmit infections.

Lapses in biosecurity practices identified were addition of new animals to the herds (73.7%, 132/179), the use of communal grazing (24.0% (43/179) of herds using with 28% (12/43) using more than one communal pasture where

mating occurred (93%, 40/43) with bulls from other herds. During communal grazing, contact herds ranged between 1 and 25 (mean = 7.4). Large herds (≥ 111) animals were more likely to use communal pasture compared to medium sized or small herds (≤ 46) ($P < 0.01$).

Domestic and wild canids had access to stored grain in 19% (34/179) of herds. The odds of wildlife gaining access to stored grain is twice as high (OR=2.37, $P < 0.02$) in western Canada compared to eastern Canada.

Purebred herds were less likely to be fed on the ground compared to cross-bred herds ($P < 0.03$). Herds from western Canada administered more feedstuffs on the ground compared to herds from eastern Canada ($P < 0.01$). Large herds were more likely to store feedstuffs outdoors compared to small herds ($P < 0.01$). Herds from western Canada were more likely to store their feedstuffs outside compared to herds from eastern Canada ($P < 0.01$).

The odds of not removing surface manure from maternity pens was almost three times (OR=2.98, $P < 0.01$) in herds from western Canada compared to eastern Canada. 78.7% (140/179) of herds disposed of manure by spreading on surface ground. 52% (93/179) of herds borrowed manure contaminated equipment from other producers for use on their farms.

Thirty three percent (53/179) of herds performed breeding soundness examinations in breeding bulls and 9.5% (17/179) of herds performed trichomonas testing on breeding bulls. Large herds were more likely to co-mingle cows and

heifers during the breeding season ($P<0.01$) compared to small herds. Cow-calf pair separation from other cows after calving occurred more in large herds compared to medium sized or small herds ($P<0.01$). The odds of using maternity pen as hospital pen was twice in western compared to the eastern Canada ($OR=2$, $P=0.04$).

Sixty percent (107/179) of herds used the same area for calving and winter-feeding. Herds from western Canada were more likely to use the same area for calving and winter feeding compared to herds from eastern Canada ($P<0.01$). Forty-one percent (73/179) of herds used hospital pens as maternity pens during calving season. 35.8% (64/179) of herds transported animals to veterinary clinic for treatment. In nine percent (16/179) of herds visitors or outside employees changed their boots, and in 18% (32/179) visitors washed their boots.

Eighty two percent (146/179) of herds dehorned cattle, of which 74% (109/148) used non-bloodless methods. Of herds using non-bloodless dehorning methods, 28.4% (31/109) disinfected dehorning equipment between animals. 73.7% (132/179) of herds reported castrating animals, of which 32.6% (43/132) used surgical castration method.

Of herds using surgical castration method, 81.4% (35/43) disinfected surgical equipment between animals. 12.2% (22/179) of herds disinfected or used new needles between animals when injecting drugs or vaccines. Thirty five percent (63/179) of herds changed sleeves between animals when performing rectal

examinations. Over twenty nine percent (29.6%, 53/179) of herds left abortions for dogs and coyotes while 27.4% (49/179) of herds left stillbirths for dogs and coyotes. The risk of adding calves persistently infected (PI) with Bovine Viral Diarrhea (BVD) virus exists with 13% (23/179) of herds adding unweaned beef calves and 18.4% (33/179) adding weaned beef calves without pre-purchase testing. PI calves are known for shedding large amounts of BVD virus and spreading BVD virus infection in beef herds.

BVD virus vaccination may compensate for exposure to the virus in a cowherd by mitigating the risk of fetal infection; however, the timing of vaccination is essential to offer protective immunity. BVD virus vaccine administered during pregnancy check may not protect the fetus against BVDV infection. There is likelihood of infection in-utero resulting in immunotolerant fetus persistently infected (PI) with BVDV and carried to term. This may occur if calf was infected in-utero before 125 days of gestation. Herds in this study administered BVD virus vaccination to breeding cows prior to breeding (60%, 118/179), during pregnancy check (28%, 50/179), and at other times (6%, 11/179).

In replacement heifers, BVD vaccination was administered prior to breeding (79%, 142/179), during pregnancy check (13.4%, 24/179), and at other times (7.3%, 13/179). Herds vaccinating breeding cows for BVD virus were 63.1% (113/179), of which 29.1% (52/179) used modified live vaccine and 34.1% (61/179) used killed vaccine. Herds vaccinating replacement heifers for BVD were

sixty percent (107/179), of which 25.1% (45/179) used modified live virus vaccine and 34.6% (62/179) used killed vaccine.

The role of the veterinarian is essential in educating producers on what constitute risky practices and how to mitigate such risks. Approach to mitigating risks may not necessarily be the same for all cow-calf herds; it must be tailored to each production unit. Initial risk assessment will identify what constitutes risky management practices, after which sound mitigation measure are designed to address such risks. On-farm biosecurity practices needs approach within the framework of risk assessment and periodic review for effectiveness.

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DEDICATION

To Adesola, my wife and our three wonderful children: Ayomikun, Kayode and Olamide.

"There is none like the God of the righteous,
He will ride the heavens to help me,
His Excellency and glory is far above the clouds.
The eternal God is my refuge, and His
Everlasting arms will always hold me high".
(Exodus 33:26-27a, NKJV paraphrase)

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1. INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

This work was based on a Canada-wide cross-sectional prevalence study of *Neospora caninum* (NC) and Enzootic Bovine Leukosis (EBL) in beef cow-calf herds in Canada in 2002 and 2003 (CFIA, 2002). The aims of this study were to determine the herd-level seroprevalence estimate of NC and EBL, identify risk factors contributing to seroprevalence, and assess biosecurity practices related to management practices in beef cow-calf herds.

This cross-sectional seroprevalence study involved collection of serum from a representative population of cow-calf herds in Canada. Surveillance data generated from this study will serve many purposes: provision of seroprevalence estimates, suggest means for disease control and will provide a baseline reference for future studies.

The diseases under investigation are infectious and chronic in nature with lifelong infection. *Neospora caninum* and Bovine Leukemia Virus (BLV) infection are endemic in the beef cattle population in Canada as confirmed by previous prevalence studies (Waldner et al., 1998; Samagh and Keller 1982).

Although NC prevalence studies carried out separately in beef cattle in Canada have reported different prevalence; there is not a single study carried out at the national level to quantify the prevalence of NC. The importance of NC lies in the ability of the pathogen to cause reproductive failure in cowherds with clinical

signs of abortion, stillbirth and embryonic death. Although, the characteristic pattern of abortion caused by NC in cowherds is sporadic, this may suddenly turn into epidemics of abortion storms without predictive signs.

The last national prevalence study on BLV in Canada dated back to 1982; no current data exist on BLV prevalence in beef cattle (Samagh and Keller 1982). BLV is not a zoonosis and of no major economic importance, but it is of concern in international trade of live cattle, bovine semen and embryos

Currently, no data exist on biosecurity practices in beef cow-calf population in Canada and reports are scarce on studies examining biosecurity aspects of farm management practices in beef cow-calf herds. These management practices include breeding, calf and calving, feeds and feeding, veterinary procedures and vaccination practices.

Lapses in management practices may result in biosecurity risks allowing introduction of disease into a herd and causing increased morbidity or mortality, decreased productivity, decreased reproductive efficiency and the loss of marketing options (Anderson et al., 1998; Dargatz et al., 2002).

Since this is a cross-sectional study, we cannot establish temporal relationships, as such; cause and effect inferences are difficult to make with certainty (Dohoo et al., 1994).

1.2 REVIEW OF LITERATURE

1.2.1 *Neospora caninum* review of literature

1.2.2 Introduction

Neospora caninum is an intracellular cyst forming protozoan parasite and manifests primarily as a cause of abortion and other reproductive problems in herds of infected cattle (Dubey et al., 2006a; Hall et al., 2005; Waldner et al., 2001b, 2005).

Neosporosis has been reported in beef and dairy herds all over the world including Canada and the United States (Barling et al., 2001; Bergeron et al., 2000; Keefe and VanLeeuwen, 2000; Sanderson et al., 2000; Scott et al., 2006; VanLeeuwen et al., 2006; Waldner et al., 2001a).

1.2.3 Description, life cycle and host range

Neospora caninum belongs to the phylum Apicomplexa, with three infectious stages – tachyzoites, tissue cyst (bradyzoites) and oocysts. The domestic dog and coyote are definitive hosts of *N. caninum*, while intermediate hosts are herbivores. Tachyzoites and tissue cysts occur in the intermediate hosts, herbivores, and oocysts occur in the definitive host (Dubey et al., 2007; Gondim et al., 2004; Lindsay et al., 1999).

Tachyzoites measure about 6x2 µm, while tissue cysts measure about 107 µm and are both localized in the nervous system. The tissue cyst wall has a

thickness of about four μm and bradyzoites measuring about $7 \times 2 \mu\text{m}$ are located in other tissues (Dubey et al., 2007). While tachyzoites occur in nervous tissue, there is no evidence in the literature they occur in the testes.

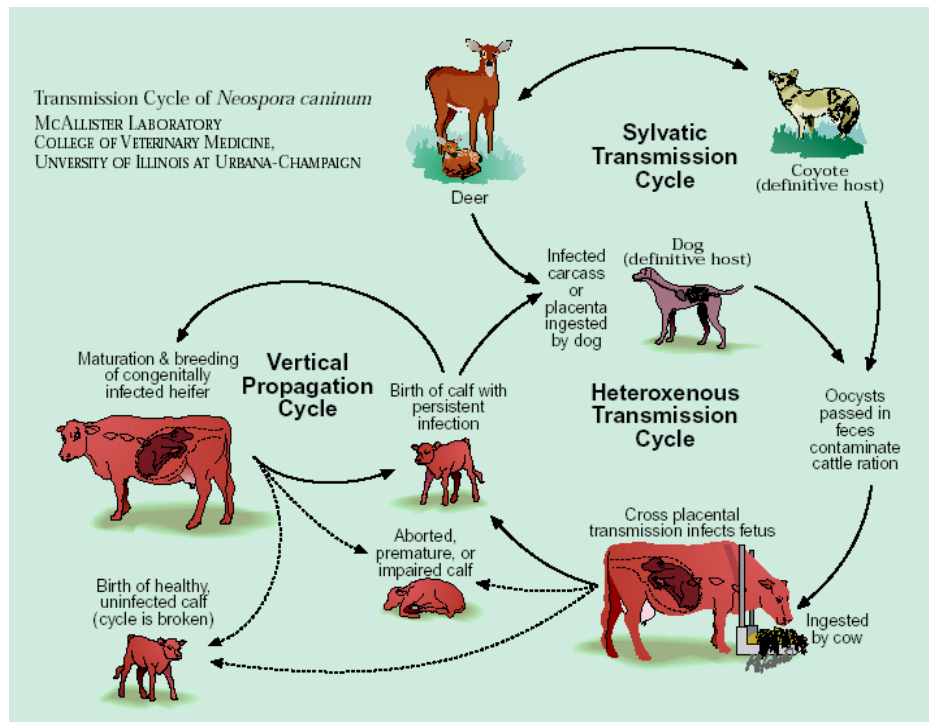


Figure 1: Transmission cycle of *Neospora caninum*

Neospora caninum and *Toxoplasma gondii* are separate and distinct parasites, despite initial suspicion of relatedness (Fazaeli et al., 2000).

Neospora caninum definitive hosts excrete unsporulated oocysts into the environment where sporulation occurs after about 24 hours. Intermediate hosts (herbivores) ingest infectious oocysts after sporulation at watering points or on pasture after which sporulated oocysts penetrate the walls of the intestine.

Neospora infections was reported in cattle, sheep, goats, llamas, alpacas, horses, water buffalo and white tail deer, cats, camels and pigs (Dubey et al., 2007).

1.2.4 Transmission of *Neospora caninum*

Although *N. caninum* infection in cattle herds appears to be mostly maintained by vertical transmission via transplacental route (Pfeiffer et al., 2002; Waldner et al., 2001b), horizontal transmission has also been suggested to play a role in *Neospora* infections (Bergeron et al., 2000; Dijkstra et al., 2001).

Neospora caninum infected dogs are potential sources of horizontal transmission in cow herds and a cyclical pattern of infection has been suggested to occur (Dijkstra et al., 2001; Gondim et al., 2002). Point-source exposure of cattle to infective oocysts from canids at feeding and watering points contribute to new *Neospora* epidemics (McAllister et al., 2000).

A recent study concluded that natural post-natal infection in heifers may lead to endogenous transplacental transmission in their offspring (Dijkstra et al., 2002). Both cyclic transmission of *Neospora caninum* in dogs and sylvatic transmission cycle between wild and domestic animal occurs in the propagation of neosporosis in cattle (Wouda et al., 1999; Schares et al., 2001; Gondim et al., 2004).

1.2.5 Clinical signs

1.2.5.1 Infection at different stages of pregnancy

In pregnant heifers and cows, immunological responses elicited in *N. caninum* infection are both cellular and humoral in experimentally and naturally infected animals (Andrianarivo et al., 2005; Moore et al., 2005). Both cellular and humoral immune responses offer effective protection against abortion (De Maretz et al., 1999; Innes et al., 2002; Williams and Trees 2006).

Depending on the development of the immune system of the fetus, seroconversion, fetal death and resorption, abortion, or birth of a weak calf may occur (Williams et al., 2000 and 2007; Rosbottom et al., 2008; Lopez-Gatius et al., 2007; Gibney et al., 2008, Bryan et al., 1994).

In immunologically naïve non-pregnant cow or heifer, *Neospora* infection usually produces no clinical signs but seroconversion occurs and the development of cell-mediated and humoral immunity (Innes et al., 2002). Infection occurring early in early gestation (\leq 2-3 months), usually induces a Th1-type immune response (Williams et al., 2007; Rosbottom et al., 2008, Maley et al., 2006), which may interfere with pregnancy and may lead to abortion (Williams et al., 2000, Innes et al., 2002; Lopez-Gatius et al., 2007).

However, in mid gestation (3-5 months), the immune system of the fetus is not yet completely developed. Infection with *Neospora* may result in the birth of a

weak or abnormal calf, encephalomyelitis or malformation of the CNS (Bryan et al., 1994; McIntosh and Haines, 1994)

In late gestation, the fetus is immune-competent, and may withstand *Neospora* infection. In addition, this may result in a switch to Th2-type immune response at the placenta; which may favor cross placenta transmission of *Neospora* (Innes et al., 2002, 2005, Maley et al., 2006). Due to immune competence of the fetus at this time, infection may result in the birth of a clinically normal or the birth of a congenitally infected calf (Williams et al., 2000; Gibney et al., 2008).

IFN- γ has a protective role against abortion in infected cows (Innes et al., 2002; Lopez-Gatius et al., 2007). A recent study suggested that Th1 immune response, in which IgG2 antibodies prevail may be protective against *Neospora* induced abortion in the presence of IFN- γ production. Elevated IgG2 antibody titers appear to be insufficient in protecting dams chronically infected with *N. caninum* against abortion (Almeria et al., 2009).

1.2.5.2 Neonatal calves

In neonatal calves, *N. caninum* infection occurs via the oral route followed by seroconversion. IFAT and ELISA detected *Neospora*-specific IgG1 and IgG2 antibodies in serum from infected calves within 2 to 4 weeks post infection but not from control calves (De Marez, et al., 1999).

In a follow up study to assess pre-weaning performance in calves born after an epidemic of abortion in which 85% (153/180) cows were positive for *N. caninum*; 67% (75/112) samples collected from calves prior to receiving colostrum had antibody titers to *N. caninum*, using an ELISA with a sensitivity and a specificity of 98.6% and 98.9% respectively.

Absence of precolostral antibody may not always reflect infection status in neonatal calves. Calves infected *in-utero* prior to developing immune competence may not develop antibodies detectable at the time of birth (Waldner et al., 2002).

1.2.5.3 Reproductive problems

An extensive study evaluating the long-term impact of neospora abortion on reproductive performance conducted in a Canadian beef herd described the pattern of infection and abortion. The herd was monitored using serology with examination of individual calving and abortion records. Approximately 81.3% of bred cows (282/347) and 86.7% of heifers (85/98) were serologically positive for *Neospora caninum* antibodies.

In the following spring, 49.2 % (98/199) of cows and 47.1% (48/102) heifers remained seropositive for *N. caninum* in the first breeding season following the outbreak, and 22.2% of cows and 13.5% of heifers were not pregnant. Animals that remained serologically positive in the next spring after the epidemic were less likely to be pregnant in the fall, OR=2.0 (95% CI: 1.1- 3.7), (Waldner et al., 2001b).

During a *Neospora* outbreak investigation in the United States, mean antibody concentration on day 14 in cows was 0.57 ± 0.33 , using an ELISA test, and this decreased to 0.46 ± 0.28 on day 71 of the epidemic. In the same study, an inverse relationship existed between antibody concentration and IgG avidity (binding strength), using an ELISA IgG avidity test. Antibody concentrations decreased significantly, but mean (\pm SD) avidity value increased significantly on day 85 (42.6 ± 14.5) into the outbreak compared to mean avidity value on day 14 (30.1 ± 16.5). This study suggested the usefulness of IgG avidity in determining chronic infections in the cowherd (McAllister et al., 2000).

While both studies conducted *Neospora* outbreak investigations; study design, aims, and conclusions were different. The first study set out to investigate and established that reduced reproductive performance occurs following a *Neospora* epidemic and seropositivity is a risk for subsequent abortions (Waldner et al., 2001b).

The second study set out to investigate and established that IgG avidity increases with chronicity of infection. The authors also suggested that immunity from previous *Neospora* infections might be protective for abortions in a *Neospora* epidemic when compared to very recent infections before the epidemic (McAllister et al., 2000).

In an earlier *Neospora* outbreak investigation, the authors had concluded that cows with high antibody titers were at greatest risk of abortion, and

seropositivity was a risk for abortion (McAllister et al., 1996). American researchers in another *Neospora* outbreak investigation described the same finding observed in the Canadian study. They found that seropositive cows were 6 times (OR=6.2) more likely to not be pregnant compared to seronegative cows, and there were high numbers of seropositive animals during a *Neospora* epidemic, (81.3% 282/347), (Waldner et al., 1999).

1.2.6 Seroprevalence of *N. caninum* in North America

Several serological studies described below on *N. caninum* in beef cattle showed different seroprevalence. A Canadian study conducted over a 4-year period in beef herds to determine seroprevalence demonstrated associations between serologic status, rate of abortion, stillbirth, calf mortality and reproductive failure. The risks of abortion (OR=5.7) and stillbirth (OR=2.8) in seropositive cows were significantly greater than in seronegative cows. Although the percentage of seropositive samples varied from 27% (51/189) to 15.9% (27/170) over the 4-year period, thirty percent (30%) of cows were seropositive at some point (Waldner et al., 1998).

A study reported overall seroprevalence of 23% (95% CI: 23-27) in beef cows from the northwestern United States and an association between seroprevalence and higher winter stocking density was demonstrated (Sanderson et al., 2000). Another study carried out a spatial analysis of seropositivity and

showed 131 steers on 54 of 94 ranches in Texas testing positive. This study demonstrated associations between seroprevalence, cattle density and abundance of gray foxes, coyotes or both (Barling et al., 2000).

In a random study of 4 feedlots with multi-sourced bull calves and steers from British Columbia, Alberta, Saskatchewan, and Manitoba; 128 of 1976, 6.5% (95% CI: 5.1-8.2) tested positive for antibodies to *N. caninum* (Waldner et al., 2001a). Comparing this study to a similar study in the US, 13% of 1009 Texas beef calves tested positive for antibodies to *N. caninum* (Barling et al., 2000),

Another Neospora risk factor study carried out in Texas sampled 760 calves from 76 herds. Spring calving, stocking density > 1 cow/calf unit/2.2 ha, wildlife access to the weaning supplement, and use of a round-bale feeder were identified as risk factors for seropositivity. Ranches self-rearing replacement heifers also had an increased risk for seropositivity. However, the use of working dog and the use of a self-contained feeder for cow supplements were associated with a reduced risk for seropositivity (Barling et al., 2001).

Different serological assays used might be a reason for differences observed in seroprevalence between Canada and United states. Another possible reason was inclusion of *Bos indicus* and *Bos taurus* cattle in United States study. Since the genetic composition of *Bos taurus* and *Bos indicus* are different so would their reaction to the same diagnostic test. The majority of cattle in North America are of *Bos taurus* origin on which the serological assay used in this study

was validated (Barling et al., 2000; Sanderson et al., 2000; and Waldner et al., 2001a, 2004).

Recently in Canadian beef cattle, a large study found associations between serological status for *N. caninum* and pregnancy status, and risk of subsequent abortion or stillbirth. In a recent study, approximately 5.9% (128/2484) of cows tested positive for antibodies to *N. caninum*. The odds of a seropositive cow not being pregnant was almost twice ($OR_{\log S/P}$, 1.9; 95% CI: 1.2–2.9) and the odds of abortion in a seropositive cow was almost thrice compared to a seronegative cow ($OR_{\text{pos/neg}}$, 2.8; 95% CI: 1.1–7.5), (Waldner, 2005).

In endemic herds, Neospora infection is usually at low prevalence with seropositive cows being at an increased risk of abortion, stillbirth and increased risk of culling. However, only a small proportion of seropositive cows abort in endemic neosporosis.

While high seroconversion rate may be an indicator of impending Neospora epidemic, high seroconversion rate without an increase in the rate of abortion occurred in a cow population (Dijkstra et al., 2002). In a herd experiencing sporadic abortion due to neospora, test and removal of seropositive cows may be a practical option.

Majority of congenital Neospora infections do not lead to abortion in cowherds and *N. caninum* oocyst may be isolated from the placenta of seropositive cows giving birth to full term calves (Bergeron et al., 2001). The trigger that turns

endemic to epidemic neosporosis is still unknown, as is the role played by horizontal transmission in *Neospora* epidemics.

1.2.7 Risk factors for bovine neosporosis

Regional differences, different management systems and presence of potential risk factors have been attributed to varying seroprevalence of *N. caninum* (Mainar-Jaime et al., 1999; Scahres et al., 2003). Several risk factors have been associated with *N. caninum* seroprevalence; these include presence and number of dogs (Bartels et al., 1999), feeding pooled colostrum to calves (Mainar-Jaime et al., 1999), rabbits and poultry on the farm (Trees et al., 2000), stocking density (Sanderson et al., 2000), and seasonality (Thurmond et al., 1995).

In risk factor studies, the presence of farm dogs suggested was a risk factor for seropositivity to *Neospora* in cattle (Bartels et al., 1999; Pare et al., 1998; Wouda et al., 1999). However, in another risk factor study, the presence of dogs reduced the risk for seropositivity to *N. caninum*.

The study concluded that the presence of working dogs might reduce seropositivity to *N. caninum* by keeping other canids away from contaminating feed and water (Barling et al., 2001). However, no data exists on proportion of herds using working dogs.

1.2.8 Diagnosis of *Neospora caninum*

Many diagnostic tests developed to detect *N. caninum* are routinely used. These include immunohistochemical (IHC) staining in the brain and fetus (McIntosh and Haines 1994), indirect fluorescent antibody test and histopathology (IFAT) (Dubey et al., 2006b), enzyme-linked immunosorbent assays (ELISA) (Bjorkman et al., 2003), direct agglutination tests (DAT) (Packham et al., 1998) Western blot (WB) analysis (Alvarez-Garcia et al., 2002), and polymerase chain reaction (PCR) (van Maanen et al., 2004).

1.2.9 Control of *Neospora caninum*

The approach to controlling *Neospora caninum* will depend on whether disease is endemic or if herds are free of disease (Hall et al., 2005). In herds free of disease, the aim is to prevent exposure to *Neospora* oocyst, which may be almost impossible; serological monitoring and removal of seropositive cows is a viable control option in disease free herds.

Efforts to investigate every case of abortion and reproductive problems in the herd are essential to rule out neosporosis. However, most cases of abortions remain undiagnosed (Anderson et al; 2000). Maintaining a closed herd and purchase of replacements heifer from seronegative herds are sound disease mitigation options. Evidence in the literature is not conclusive on the possibility of bulls infecting cows and heifers. Breeding *Neospora* infected bulls to serologically

negative heifers; a single report concluded it is unlikely for venereal transmission to occur under natural grazing condition (Osoro et al., 2009). A single report detected *Neospora* in the semen and blood (Ferre et al., 2005). However, *Neospora* infected semen has not been reported to infect cows and heifers

Monitoring of range pasture for contamination with oocyst of dogs and wild canids can be challenging and virtually impossible in an extensive beef operation. Zero grazing is not a practical option in beef cattle herds, as such; serological monitoring of cattle and all farm dogs and removal of seropositives are potential control options.

In infected herds, seropositivity to *N. caninum* is a risk for abortion, but not all-seropositive cows abort, and not all calves born to seropositive dams are seropositive. Although high antibody titer may be a predictor for *neosporea* abortion, however, not all seropositive cows abort (Bergeron et al., 2001 and Dijkstra et al., 2001).

Reduction in prevalence over time is dependent on the dynamics of loss and addition to the cowherd. The choice of appropriate serological tests with high sensitivity and specificity to correctly quantify seroprevalence is the first approach. Secondly, reducing prevalence by selective culling of seropositives, proper disposal of aborted fetus and placenta in infected herds may further contribute to reducing overall prevalence.

Test-and-cull has been shown to be highly efficacious under field conditions in herds with low prevalence frequently monitored serologically to detect newly seroconverted cows which are excluded from the herd. New infections in the herd are thereby limited to vertical transmission. It was also shown that prevalence can be further lowered by breeding only seronegative heifers and cows while culling seropositive heifers and cows (Hall et al., 2005).

Since the trigger that turns endemic to epidemic neosporosis is unknown, risk factor identification and prevention may prevent endemic neosporosis from turning into epidemic neosporosis in infected herds. Preventing the access of farm dogs to aborted fetus and placenta may prevent heavy parasite burden in farm dogs, interrupt the lifecycle of the parasite and prevent dogs from shedding large amounts of infectious oocysts contaminating the pasture (Dijkstra et al., 2001a).

Replacements and restocking with proven seronegative heifers and cows may reduce herd prevalence. An economic model based on decision tree analysis suggested that it might be economical to live with the disease in an infected herd until within-herd prevalence exceeds a threshold of 18% (for a short-term of 1 year) or 21% (for a long-term of over 5 years), (Reichel and Ellis, 2007).

Use of vaccination may be another control measure. During the evaluation of a killed vaccine for use in cattle, preliminary trials suggested the vaccine might induce protection against abortion (Choromanski and Block, 2000; and Williams et al., 2003). The only commercially available vaccine against *N.*

caninum(Neoguard[®], Intervet) contains inactivated tachyzoites. Vaccine administration was prior to breeding or early in the first trimester of gestation, twice, 4 weeks apart, with one or two annual booster vaccinations.

Vaccination efficacy is limited with an abortion reduction rate of about 50% (Reichel and Ellis, 2008). In a recent study, inoculation with *N. caninum* surface antigen followed by a booster dose elicited an immune response similar to infection with live Neospora antigen in cattle (Baszler et al., 2008).

The possibility of a live vaccine offering protection against fetopathy was demonstrated in a cattle population. Since killed vaccines elicit primarily a humoral immune response, perhaps a live vaccine might elicit better cellular immune response miming natural infection and delivering a more protective immunity (Williams et al., 2007).

1.3 Bovine Leukosis Review of the Literature

1.3.1 Introduction

In 1871, one of the earliest reports described Bovine Leukosis as a lymphoproliferative disease characterized by yellowish nodules in the enlarged spleen of a cow (Leisering, 1871). Occurrence of the disease in cattle herds triggered the term enzootic Bovine Leukosis to describe permanent presence in the herd while producing clinical disease in a small proportion of animals.

Two forms of Bovine Leukosis described in the literature are enzootic Bovine Leukosis and sporadic Bovine Leukosis. The etiology of enzootic Bovine Leukosis is an oncovirus of the family Retroviridae. The etiology, transmission and pathogenesis of sporadic Bovine Leukosis are unknown.

Bovine Leukosis transmission occurs mainly by horizontal contact with infected cells. If infection is established, it is persistent and lifelong. Tumor formation and spleen disruption are the most common clinical signs of Bovine Leukosis in adult cattle. Detection of antibody to the virus has contributed to effective control and eradication efforts in cattle populations.

Bovine Leukosis occurs in cattle populations across the world including Canada and the United States. Control and eradication programs in Europe, Australasia and Oceania have been largely successful and drastically reduced disease prevalence because the control programs were regulatory and mandatory

under government supervision. Denmark was the first country to eradicate BLV in their cattle population (Flensburg and Streiffert; 1977).

1.3.2 Retroviruses and Bovine Leukemia Virus

Bovine leukemia virus (BLV) belongs to the deltaretrovirus genus, retroviridae family with related human T-cell lymphotropic virus type 1 and II (HTLV-I and II) that causes adult T-cell leukemia. Retroviruses are RNA viruses characterized by persistent, permanent and lifelong infection of host cells (Sagata et al., 1984).

Although the viruses are related, the pathogenesis and mechanism of producing tumors is entirely different. While BLV produces B-cell proliferation and neoplasia, HTLV produces T-cell neoplasia. BLV and HTLV have no preferred integration sites in tumor cells and no oncogene in their genomes. The genomes of BLV and HTLV have an X region located between the *env* region and the 3' long terminal repeat (Mamoun et al., 1985; Rice et al., 1987).

1.3.3 Bovine leukemia virus

BLV viral particle ranges from 60 and 125nm in diameter with a nucleoid bounded by a lipid envelope. The genome consists of *gag*, *pol* and *env* genes for synthesis of viral particle and an X region peculiar only to retroviruses. Lack of detection of viral transcripts in tumor and infected cells is peculiar to BLV. The

BLV genome has been described as unique and genomically stable with minimal 6% divergence when comparing worldwide BLV strains and using the pol gene as a landmark (Dube et al., 1997).

1.3.4 The Host

The natural host for BLV infection is domestic cattle (*Bos taurus* and *Bos indicus*). Natural infection occurs in water buffalo (Meas et al., 2000). BLV is not a zoonosis and human consumption of milk from infected cattle may cause seroconversion not leukemia (Burmeister et al., 2007; Perzova et al., 2000).

Experimental transmission of BLV infection occurred in sheep, goats, pigs, chickens, rats and rabbits (Mammerickx et al., 1981 and 1987; Olson et al., 1981; Altanerova 1989, 1990, 2004; Zhao et al., 2005). BLV infection also occurs in chimpanzees, rhesus monkeys and buffalo (van den Marten and Miller 1976; Schodel et al., 1986; Persechino et al., 1984). Genetic susceptibility may be a contributory factor to BLV prevalence in some breeds of cattle when compared to others (Lewin and Bernoco, 1986).

1.3.5 Transmission of BLV

Transmission of BLV occurs via transfer of infected cells (Ferrer and Piper 1978). Transmission is mostly horizontal by direct contact. A small proportion of

infections may propagate vertically via the maternal-fetal transmission route (Meas et al., 2002; Lassauzet et al., 1991; Agresti et al., 1993).

Median age of infection was estimated at 4.6 years, (Monti et al., 2007), while median time from infection to seroconversion was estimated at 47 (95% CI: 39-55) days to 57 (95% CI: 49-75) days (Monti et al., 2005). Body fluids examined and having the potential for transmitting BLV include milk, blood, bronchiolar lavage, saliva, nasal secretion and semen fluid (Konnai et al., 2006; Mammerickx et al., 1987; Roberts et al., 1982a; Ressang et al., 1982; Lucas et al., 1980; Lucas et al., 1993).

The potential for the spread of EBL through bovine semen have been investigated due to concerns in international trade. A study involved 207 embryos from BLV-infected donors sired by BLV infected bulls. Washed embryos were transferred to recipients confirmed uninfected. None of the resulting 50 calves or the recipients developed BLV- specific antibodies (Hare et al., 1985)

A similar study involved exposure to BLV during *in vitro* fertilization with 170 matured oocytes, semen were washed with BLV cell-free suspension before it was used for fertilization. BLV was not isolated from the embryos in this study and the embryos did not test positive for BLV. The authors concluded the risk of infecting embryos fertilized *in vitro* with semen from BLV infected bulls was negligible (Bielanski et al., 2000; Wrathal et al., 2006).

Successful routes of BLV transmission reported in the literature are intravenous, intramuscular, intradermal, intratracheal, intrauterine, and subcutaneous (Ungar-Waron et al., 1999, Miller et al., 1972, Evermann et al., 1986; Roberts et al., 1982b). Routine livestock management procedures in cattle have the potential risk for transmission of BLV. The possibility of iatrogenic infection exists through multiple uses of surgical blades for castration, multiple uses of tattoo equipment and dehorning methods (gougers, spoon, guillotine, and saws).

Iatrogenic infection may also occur through multi-use and/or multidose syringe and needles, and multiple uses of a common sleeve for rectal palpation (Di Giacomo et al., 1987; 1985, Lassauzet et al., 1990b, Wilesmith et al., 1990; Brigting and Radostits, 1983; Kohara et al., 2006; Divers et al., 1995).

1.3.6 Pathogenesis of BLV infection

Present knowledge of BLV infectivity was gained from experimental sheep models that showed the pathogenesis of infection is similar in sheep and cattle. Sheep are not natural host of BLV and the disease in sheep is different from cattle, however, sheep model provides for shorter latency period and disease progression compared to the cattle (Florins et al., 2008). Willems et al., 2000 concluded that leukemia in sheep is entirely due to injection of BLV provirus.

During infection, transmembrane protein destabilizes the host cell membrane with a fusion peptide, after which a structural protein enhances cell fusion, viral fusion and enhancing infectivity of host cells (Gatot et al., 2002; Alber et al., 1993). Post-infection synthesis of DNA molecules is achieved by reverse transcription despite the fact that Reverse transcriptase is commonly inhibited *in vivo* by sera containing antibodies from some leukemic cattle (Gillet et al., 2007). Structural genes – *tax*, *pol* and *env* are essential to infectivity *in vivo* but deletions prevent infectivity (Willems et al., 2000).

BLV replicates by mitotic division of the infected cell. Viral DNA molecules occur in asymptomatic and persistent lymphocytosis (PL) cattle but is absent in tumor phase (Tanaka et al., 1998). The provirus, a double stranded DNA is inserted at random sites into the host genome in a process directed by integrase, the newly inserted provirus is bordered by direct repeats of host cellular DNA (Tanaka and Komuro, 2005). Deletions in the R3 and G4 genes are employed in the production of live attenuated vaccine for BLV infection (Reichart et al., 2000).

1.3.7 Clinical signs

Two types of clinical manifestations of leukosis exist in the literature. Enzootic Bovine Leukosis and sporadic bovine leukosis occur in nature. The latter is not transmissible (Ogawa et al., 1986). BLV causes B-lymphocyte proliferation

referred to as leukemia in the blood stream, lymphoma in the lymph node and lymphosarcoma in various organs (Willems et al., 2000).

Persistent lymphocytosis (PL) is the hallmark of BLV-induced leukosis; characterized by a permanent increase in the number of B-lymphocytes in the peripheral blood (Meas et al., 2002). The PL stage affects approximately one third of infected animals, PL is a result of an accumulation of untransformed B-lymphocytes in blood circulation. Viral infection with BLV is asymptomatic, and less than 1 % of peripheral blood cells in animals be infected by virus (Ferrer et al., 1979; Willems et al., 1999; Meas et al., 2002).

Development of lymphosarcoma occurs between 4 and 8 years of age in about 1-5% of infected cattle. The reason for a higher prevalence of tumors in dairy cattle compared to beef cattle is unknown; different husbandry systems and genetic predisposition have been suggested (Lewin and Bernoco., 1986). In cases of tumors caused by BLV, locally transformed B cells infiltrate vital organs such as liver, heart, eye, skin, lung and lymph nodes.

In BLV infections, cell free virus occurs *in vivo* with leukemogenesis and tumor formation mostly occurring in absence of viral expression (Kettmann et al., 1985; Ferrer et al., 1979; Wu et al., 2003). Depending on the body organs infiltrated by tumor cells, a variety of clinical symptoms are seen in affected animals. Rectal palpation of internal lymph nodes may reveal lymphadenopathy.

Organs mostly affected are vital organs: the liver, heart, eye, skin, lung, uterus, kidney and spinal canal (Willems et al., 1999).

Tumors in the hind limbs may lead to progressive hind limb paresis, a frequent reason for culling cows. Ante and post mortem condemnation due to lymphosarcoma is a common occurrence in cull cows during late fall and winter, condemnations are more predominant in cull dairy cows compared to cull beef cows (personal communication – Dr. Dennis Kirk).

1.3.7.1 Cellular and humoral response

Initial polyclonal increase in the population of lymphocytes precedes a monoclonal response during which provirus integration and genetic sequence deletion may occur. Point deletions producing ‘dead end viruses’ unable to replicate *in vivo* or large deletions ‘mutations’ may occur. BLV tumors may occur with or without insertion of deleted provirus into the host genome (Debacq et al., 2004; Moules et al; 2005; Tajima et al., 1998).

In BLV infections, leukemogenesis occurs in the absence of viral expression; as such, BLV viral proteins are undetectable in peripheral blood by routine diagnostic techniques except by Real time (RT-PCR) amplification of viral transcripts from peripheral blood or tumors (Gaynor et al., 1996). BLV provirus is exogenous with random viral gene expression due to random integration and does not produce cell transformation by insertion mutagenesis (Kettmann et al., 1980).

In vivo, demonstration of viral expression is challenging in BLV infections due to low level of viral expression. Cell sorting using flow cytometry followed by RT-PCR may yield about a ratio of 1: 10,000 B lymphocyte expressing *tax/rev* mRNA in PL. In addition, a ratio of 1:50,000 cells in peripheral blood may contain adequate BLV protein identifiable by *in situ* hybridization (Radke et al., 1992).

Cytokines in BLV infection include elevations of interleukin IL-10 from PL cows and IL-2 from lymph node T-cells and peripheral blood of infected cattle. Other cytokines elevated in BLV infections include IL-6 and IL-12 in PL in the asymptomatic cows, Tumor necrotic factor (TNF-alpha) and interferon gamma in T-cells from BLV-infected cow (Keefe et al., 1997, Amills et al., 2002, 2004; Yakobsen et al., 1998).

BLV may also infect monocytes and macrophages. Infected B-lymphocytes may express elevations of CD5 molecules. B lymphocytosis results from dramatic increases in CD5+ B lymphocytes (Matheise et al., 1992; Meirum et al., 1997). Provirus occurs in both CD5+ and CD5- lymphocytes from infected animals while lymphosarcoma cells are mostly CD5+ B lymphocyte (Saini et al., 1999).

Other BLV markers elevated in BLV infections are CD5+ and CD5-, markers CD 11b, CD11b+ and CD11b-, include IL-2 receptor, histocompatibility class II (MCH-II) complex surface IgM+ and tumor associated antigen (Wu et al., 1996; Aida et al., 1997).

1.3.8 Diagnosis

1.3.8.1 Diagnostic tests

The earliest diagnostic test used in detection of BLV was a hematologic test based on leukotic keys, which diagnosed subclinical disease on the observation of higher number of lymphocytes in blood of clinically healthy cattle from BLV endemic herds. An animal with higher lymphocyte count in adult cattle herd was leukotic when compared to other herd mates (Bendixen 1965 and 1967).

Agar gel immunodiffusion test (AGID) is the preferred test of choice for detection of BLV for international trade in live cattle as adopted by Office International des Épizootes (OIE). Sensitivity (Se) and specificity (Sp) of the official OIE AGID test was reported at 79.7% and 99% respectively (OIE 2002, Trono et al., 2001). The Se and Sp of another AGID test was 79.7 and 99.0% respectively. This was relative to polymerase chain reaction (PCR) and Southern blot (PCR-SB) used as confirmatory test (Trono et al., 2001).

Enzyme linked immunosorbent assay (ELISA) has replaced AGID for large scale testing for antibodies to BLV infection. ELISA detects gp51 surface glycoprotein and takes less time than the AGID test, usually less than 1 hour. Se and Sp of the ELISA was 97.2% and 97.5% respectively. Most ELISA test kits require the use of a plate reader thereby restricting use of the test to laboratory settings (Carli et. al., 1993).

Unlike in adult cattle, detection of viral infection in calves using serological testing is inaccurate due to interference of maternally-derived colostrum immunoglobulin. A tissue culture test called syncytium induction assay has potential dual usefulness in calves and adult cattle by detecting BLV virus in whole blood (Ferrer and Diglio, 1976).

Western Blot is a more sensitive and specific test compared to ELISA and AGID. Western blot was used as a gold standard and compared to 2 commercially available kits, agarose immunodiffusion and the enzyme immunosorbent assay. Agarose immunodiffusion and immunosorbent assay failed to detect 39% and 35% of the animals determined serologically positive by immunoblot (Choi et al., 2002). Kittelberger et al., 1999 had reported *Western blot* Se and Sp at 97.4 % and 99.4 % respectively.

Reverse transcriptase-polymerase chain reaction (RT-PCR) test uses RNA strand that is first reverse transcribed into its complement DNA using the enzyme reverse transcriptase before the resulting cDNA amplification. Se and Sp reported are 98.4% and 100% respectively (Rola and Kuzmak, 2002; Mirsky et al., 1993).

Routine use of PCR is cost-prohibitive; use of PCR is limited to research. Other diagnostic methods not routinely used for BLV diagnosis include *in situ* hybridization and flow cytometry (Gaynor et al., 1996), immunofluorescence test (Ferrer et al., 1972), radio-immunoprecipitation assays, and virus neutralization test (Jacobsen et al., 1985).

1.3.9 Seroprevalence of BLV in North America beef and dairy cattle

One of the earliest national seroprevalence studies on bovine leukemia virus in Canada was carried out in 1980. The study carried out screening on 38,297 serum samples from 990 herds of dairy and beef cattle for antibodies to BLV, using a glycoprotein antigen-antibody system and agar-gel immunodiffusion test. The overall herd seroprevalence was 19.7% while the individual animal seroprevalence was 3.6%.

Regional differences in seroprevalence observed in this study are hereby described. Herds in four Atlantic (eastern) and three western provinces had a significantly lower seroprevalence than those in Central Canada, beef herds had lower seroprevalence compared to dairy herds (Samagh and Kellar, 1982).

A survey conducted in 2000 involved 2,604 dairy cows from 89 Maritime Canada herds used random selection of dairy cattle herds in New Brunswick, Nova Scotia, and Prince Edward Island. The study randomly selected 30 dairy herds in each province and 30 cows randomly selected in each herd and tested for antibodies to bovine leukemia virus (VanLeeuwen et al., 2001).

Antibodies to bovine leukemia virus were found in 70.0% (95% CI: 60.3 - 79.7) of herds and 20.8% (95% CI: 15.8- 27.0) of cows. Positive herd criterion was having at least one seropositive cow. In BLV-positive herds, the average BLV prevalence was 30.9% (95% CI: 24.8 - 37.2), VanLeeuwen et al., 2001.

In 2001, a study in Saskatchewan used herd and animal sample size calculations similar to those used in the Maritime Canada study. A total of 1530 cows from 51 dairy herds were sampled and tested for antibodies to BL. Thirty (30) dairy cows were randomly selected in each herd and tested for antibodies against BLV regardless of herd size. Results showed 89.1% of herds had at least one animal testing positive to EBL (95% CI: 80.8- 97.4), while 37.4% of cows tested positive (95% CI: 28.8 - 46.0) (VanLeeuwen et al., 2005).

In comparing the above Maritime and Saskatchewan studies, the authors observed significant regional differences. More dairy cows (37%) and herds (89%) in Saskatchewan were seropositive for BLV when compared with dairy cows (21%) and herds (70%) in Maritime Canada. The authors concluded that there might be differences in the level of exposure and the amount of virus transmission occurring among animals in Saskatchewan and Maritime Canada. Selection bias in dairy farm sampling in Saskatchewan may have played a role in over estimating seroprevalence (VanLeeuwen et al., 2005).

Another seroprevalence study carried out in Manitoba in 1999 and 2000 sampled beef and dairy cattle from the same province. The study sampled 1425 beef cows from 49 herds and 1204 dairy cows from 40 herds. Herd seroprevalence were 47.9% (95% CI: 28.0 - 68.0) in beef herds and 97.4% (93.5 - 100) in dairy herds. Positive herd criterion was having at least one seropositive cow.

Cow seroprevalence was 21.7% (95 CI: 8.6-34.7) in beef cows and 62.5% (95 CI: 53.5- 71.4) in dairy cows. Herd and cow sample size calculation procedures were similar to those used in Maritime Canada and Saskatchewan to ensure comparability of results. There were significantly ($P < 0.05$) more BLV-seropositive dairy cattle, 60.8% (95% CI: 51.8- 69.9) when compared with beef cattle (10.3%, 95% CI: 2.5-18.0) (VanLeeuwen et al., 2006).

Based on an infected herd having at least one seropositive cow, there was significantly higher prevalence of BLV-seropositive dairy herds (97.4%, 95% CI: 93.5–100) than of beef herds (47.9%, 95% CI: 93.5–100). Furthermore, in these infected herds, the within herd seroprevalence was significantly higher in dairy herds (62.5%, 95% CI: 53.5–71.4) than in beef herds (21.7%, 95% CI: 8.6–34.7) (VanLeeuwen et al., 2006).

In a Canada-wide survey reported in 1980 (Samagh and Kellar, 1982), 40% of dairy and 31% of beef herds in Manitoba were found to be BLV-seropositive. This was considerably lower than the prevalence found in the more recent studies. Other Canadian provinces have also seen substantial increases in cow and herd level BLV seroprevalence in dairy cattle over the last 20 to 25 years (VanLeeuwen et al., 2001; VanLeeuwen et al., 2005).

A study carried out on BLV seroprevalence in Alberta dairy herds during 2002 and 2003 used the same study design and sampling previously employed in Canadian studies in dairy herds (VanLeeuwen et al., 2001, 2005).

Using random selection, 2,814 dairy cows from 77 herds were sampled and tested for antibodies to BLV, herd seroprevalence was 86.7% (95% CI: 78.5- 92.1) and individual animal seroprevalence as 26.9% (95% CI: 22.1 - 32.2). A positive herd had at least one cow testing positive. BLV seroprevalence in dairy cattle herds in Alberta was similar to that estimated for other regions of Canada (Scott et al., 2006).

In the United States, a serological survey conducted in 1975 found 10.2% of the dairy cattle and 1.2% of the beef cattle testing positive for antibodies to BLV. Herd size was as a risk factor in this study. The reason why dairy herds with less than <50 cows were more likely to be positive is not known (USDA: APHIS: VS: NAHMS; 1997).

In Florida, an unbiased study estimated the prevalence of BLV in beef cattle was in 1978. The study involved 28 beef herds selected from four geographic regions by stratified random sampling procedures. Using AGID, antibody point prevalence was 7% in 4911 beef cattle. The study also found 48% prevalence in dairy cattle and a positive association between age and seropositivity in both beef and dairy cattle, more so in dairy cattle (Burridge et al., 1981).

1.3.10 BLV control and eradication

Three control methods recommended for BLV are test and slaughter, test and segregate, test and corrective management, or a combination of all the

methods (Sprecher et al., 1991; Monti et al, 2007). Test and slaughter is practical in herds with a low prevalence. Test and segregation as a control measure for BLV is not economically feasible in large commercial operations. Culling of all positive animals is also cost prohibitive in large commercial operations.

Test and segregation with corrective management reduced prevalence in a dairy herd from 95% to 34% over 3 years. Risk mitigation procedures instituted for management practices associated with transmitting BLV proved effective (Johnson et al., 1985). Test and removal with corrective management was recommended as an effective control measure in a recent study (Monti et al., 2007)

At a regional level, the initial approach to control is to quantify the prevalence of disease, which may come from national serological data identifying seropositive herds. The diagnostic test employed in screening should have a high sensitivity to detect positive herds, to ensure maximizing the detection of false negatives that could spread the disease. Initial herd screening determines infected herds after which individual cow-level test is applied to establish true BLV status (Sargeant et al., 1997).

Approach to the control of BLV infection depends on herd size and the prevalence of infection. Smaller dairy herds with low or high BLV prevalence may adopt an eradication approach through test and cull with corrective management; and restock from certified seronegative herds. Test and removal with corrective measure implementation twice in a year may reduce prevalence by 25% in small

and medium sized herds. In large dairy operations, test and segregation with corrective management may take several years to yield results. Initially, the short-term goal is test and segregation in large dairy herds, followed by selective culling of seropositive herds from groups of segregated animals within the herd.

Calf rearing management practices for replacements that prevent new infection with testing after calf hood to segregate positive animals is a recommendation for reduction of prevalence. A control program driven by industry with value-added incentives and marketing options for seronegative BLV herds may eventually reduce seroprevalence.

Testing and culling is not feasible in large commercial herds with high BLV infection. Test and segregation or test and cull programs in large commercial herds is arduous, painstaking and costly. Any lapse in strict meticulous protocol may increase seroprevalence, delay attainment of BLV-free status or result in loss of disease free status in seronegative herds.

Financial incentives may be necessary to entice large commercial operations to control BLV since there is presently very little or no incentive in being BLV free. Consideration for BLV latency and lag time from viral infection to serologic detection is very critical to the success of a control program. Viral latency may play a role in lack of detection of circulating antibody during testing for BLV, necessitating repeat testing after 3 - 6 months (Nuotio et al., 2003).

1.4 Biosecurity practices in beef cattle literature review

1.4.1 Introduction

Biosecurity practices are activities undertaken to prevent the introduction of disease agents into an area of interest. The area of interest may be at the farm level, provincial or state level, regional or national level. At the farm level, preventing introduction of an infectious disease agent, reducing transmission within the herd and preventing diseases from leaving the farm are different aspects of disease control (Anderson, 1998). On-farm biosecurity employs a variety of approaches to manage disease determinants by identifying and controlling for management practices with the potential for introducing new pathogens.

1.4.2 Addition of new animals

The addition of calves, cows and bulls into a production unit from other farms or producers may be a potential source of new pathogens in the herd. The addition of new calves may introduce Bovine Viral Diarrhea Virus (BVDV) into a herd by purchasing calves persistently infected with BVD virus (Houe, 1995).

Calves purchased from sale yards are risk factor for enteric and respiratory diseases. Especially, commingled calves from several sources of origin and exposed to a wider variety of pathogens (Fulton et al., 2005; Callan and Garry, 2002). Multiple disease agents cause enteric infections in neonatal calves, and

management practices adopted to minimize the risk of enteric infection protect for other infections (Moon et al., 1978).

1.4.3 Exposure to other herds

Venereal diseases such as trichomoniasis and campylobacteriosis can enter a herd through the addition of bulls or breeding cows. Commingling of herds in communal grazing is a significant risk factor for venereal diseases. A prevalence study of 57 of 60 randomly selected beef cow-calf herds found 4% of bulls and 16% of herds positive for *Trichomonas fetus* (BonDurant et al., 1990). Another study found T. fetus prevalence of 11.9% among bulls in a large multi-unit ranch with bull group prevalence ranging from 0% to 39% (Rae et al., 1999).

1.4.4 Feeds and feeding

Contaminations of feeding and drinking areas have been associated with outbreaks of neosporosis in beef cattle (Dijkstra et al., 2002). Shared pasture or communal grazing, contact at fairs or exhibitions, lending cow or bulls and borrowing cow or bulls may have the potential for introducing new disease into a herd. Contact between livestock, domestic and wild canids was a risk factor for *Neospora caninum* infection (Gondim et al., 2004; McAllister et al., 1996).

1.4.5 Manure management

Manure disposal by spreading manure on surface soil or injection of manure into the ground may be a potential risk factor for infectious disease in beef cattle. *Mycobacterium avium paratuberculosis* (MAP) persisted in the manure of range beef cattle and in the environment for up to 55 weeks (Whittington et al., 2004). *Salmonella typhimurium* can contaminate the soil after incorporation of contaminated bovine manure (Natvig et al., 2002).

Borrowing of manure-contaminated equipment between livestock farms may have the potential of transmitting disease. *Salmonella typhimurium*, a potential zoonotic agent survives in fecal material in dry or wet substances in the environment for 4-5yrs (Plym-Forsshell and Eksebo, 1996). The environment where pathogens survive for long periods may serve as a constant source of re-infection. This may pose a challenge to disease control and biosecurity efforts.

1.4.6 Vaccination

Experimental transmission of BVDV via contaminated vaccines has occurred in dairy cattle (Barkema et al., 2001; Falcone et al., 2003). Poor hygienic practices and environmental contamination may play a role in transmission of BVDV (Niskanen and Lindberg, 2003; Lindberg et al., 2004). The use of the same injection needle during vaccination (Wilesmith 1979; Brightling and Radostits, 1983; Lassauzet et al., 1990) and the same tattoo instrument without disinfection

has been associated with BLV transmission in dairy replacement heifers (Lassauzet et al., 1990).

1.4.7 Other biosecurity practices

Risk of infectious disease transmission has occurred when the same rectal glove was shared among animals during rectal palpation (Kohara et al., 2006). The use of hospital pens as a maternity pen in beef cowherds may predispose immunologically naïve newborn calves to infections. Animal processing practices promoting cross-contamination of body fluids between animals may have the potential for transmitting diseases. Gouge dehorning transmits BLV infection in dairy replacement heifers (DiGiacomo et al., 1985 and 1987).

Other practices promoting cross-contamination of body fluids between infected and non-infected animals includes surgical castration with a single scalpel blade without disinfection, multiple uses of ear-tag /notching equipment without disinfection and use of same needle for injecting vaccines.

Hospitalization of sick animals and returning them to the farm of origin is a potential biosecurity risk due to nosocomial infections. Manure on boots and clothing, manure contaminated vehicle tires and visits to different farms on the same day by animal health workers may have a potential for transmission disease.

Lapses in biosecurity practices may result in the introduction of disease into a new herd resulting in increased morbidity or mortality, decreased

productivity, decreased reproductive efficiency and the loss of marketing options (Anderson et al., 1998; Dargatz et al., 2002)

Traditionally, on-farm vaccination has been used as a disease control tool preventing untoward effects of pathogens in production animal population. The merits of vaccination have proven to be insufficient in preventing untoward effects of infectious disease at the farm level. As such, a more inclusive approach of all farm management practices needs consideration in pathogen prevention.

Biosecurity programs address farm-level management practices that may have the potential for introducing disease into the herd. Recent emphasis on farm-level biosecurity in production animal medicine may be due to the emergence of infectious disease problems with zoonotic potential in farm animal populations.

In conclusion, consideration of biosecurity practices should include the epidemiological triad of host, agent (reservoir /vector) and the environment. In addition, modes of transmission (direct and indirect), pathogen characteristics, environmental survivability, incubation period and period of communicability are of concern in an effective biosecurity program (Barrington et al., 2002).

1.5 Objectives

The objectives of this study were to:

- Determine the prevalence of *Neospora caninum* and Bovine Leukemia Virus in beef cow-calf herds in Canada

- Identify risk factors contributing to the prevalence of the infections.
- Identify the prevalence of various biosecurity practices in beef cow-calf herds in Canada.

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2. Neospora caninum seroprevalence in beef cow-calf in Canada

2.1 Introduction

Neospora caninum has emerged as a major cause of reproductive disease and considerable economic importance in cattle throughout North America (Anderson et al., 2000). Surveys reported an increasing proportion of aborted fetus submissions diagnosed with *N. caninum* (Dubey et al., 2005; Khadokaram-Tafti and Ikede, 2005). Epidemiologic studies have shown that seropositive cows are more likely to abort when compared to seronegative cows (Hall et al., 2005; Pfeifer et al., 2002).

Although *N. caninum* infection in cattle herds appears to be mostly maintained by vertical transmission (Pfeifer et al., 2002), horizontal transmission had been suggested to play a role in *Neospora* infections (Bjorkman et al., 2003; Djikstra et al., 2001). *N. caninum* infected dogs are sources of horizontal transmission in cowherds and a cyclical pattern of infection has been suggested (Djikstra et al., 2001; Gondim et al., 2003; Scabies et al., 2001).

Dogs fed tissues of experimentally infected calves are able to shed more than 10,000 oocysts of *N. caninum* (Djikstra et al., 2001). Seroprevalence of antibodies to *N. caninum* has been shown to be significantly higher in farm dogs when compared to dogs in urban areas (de Souza et al., 2002; Sanchez et al., 2003), suggesting the possibility of a sylvatic cycle for *N. caninum* (Gondim et al., 2004a). Antibodies to *N. caninum* have been found in wildlife and wild canids

such as foxes and coyotes are now considered natural hosts for *N. caninum* as well (Gondim et al., 2004b). However, the role of wildlife and wild canids in the epidemiology of *N. caninum* in domestic cattle is still under investigation.

Data on seroprevalence of *N. caninum* in beef cattle in North America is scarce. Reports from north-western United States showed a mean cow-level seroprevalence of 23%, with all the herds having at least one seropositive cow (Sanderson et al., 2000). In a more recent study of beef calves in Texas, 13% of the animals were seropositive with 59% of the herds having at least one seropositive calf (Barling et al., 2001).

In western Canada, two studies have reported individual seroprevalence in beef cattle (6% and 9%, respectively), in the second study a herd prevalence of 36% was reported where a positive herd was defined as at least one cow testing positive (Waldner et al., 2001 and 2005)

Regional differences, different management systems and the presence of potential risk factors have been attributed to varying the seroprevalence of *N. caninum* (Mainar-Jaime et al., 1999; Scahres et al., 2003). Several risk factors have been associated with *N. caninum* seroprevalence; these include the presence and number of dogs (Bartels et al., 1999, 2007), feeding pooled colostrum to calves (Mainar-Jaime et al., 1999), having rabbits and poultry on the farm (Trees et al., 2000), stocking density (Sanderson et al., 2000), and seasonality (Thurmond et al., 1995).

Although suggested, it is not known if the extensive husbandry and management systems usually employed in beef cow-calf herds play a significant role in *N. caninum* infection when compared to dairy herds (Quintanilla-Gozalo et al., 1995). The objectives of this study were to determine the seroprevalence for *Neospora caninum* infection in Canadian beef cow-calf herds and to identify potential risk factors associated with the herd seroprevalence.

2.2 Material and Methods

2.2.1 Study population

The study population consisted of beef cow-calf herds in the provinces of Canada: Alberta, British Columbia, Ontario, Saskatchewan, New Brunswick, Nova Scotia and Prince Edward Island. Provinces in Maritimes (New Brunswick, Nova Scotia and Prince Edward Island) were grouped as a region called Atlantic Canada. Surveys in Quebec and Manitoba were carried out independently (VanLeeuwen et al., 2006). Promotional materials were sent to Canadian beef industry associations in June 2002. Producer's recruitment for the study was done in 2003 by random sampling of 4,700 cow-calf producers in all participating provinces.

To estimate the number of cows required to be tested in each herd the following assumptions were used: average herd size of 45 cows, an expected average within-herd prevalence of 10%, allowable error of 6%, and a level of

confidence of 95%. Thirty cows per herds were thus required for testing (Martin et al., 1987).

2.2.2 Questionnaire survey

A comprehensive, 19-page mail-in questionnaire was designed to gather information on management, biosecurity and demographic factors. The questionnaire was divided into 6 different sections: farm profile, calves and calving, feeding practices, veterinary procedures and vaccination, and farm biosecurity (questionnaire available upon request – Appendix I). Information from the questionnaire used for this study is shown in Table 2.

2.2.3 Serology

During the fall of 2003, at the time of pregnancy diagnosis, blood samples were collected from randomly selected cows in each herd; serum was harvested and stored at -20°C. Testing for antibodies to *N. caninum* was done at the Centre for Animal Disease Surveillance Laboratory of the Canadian Food Inspection Agency (CFIA), Ottawa, using a commercially available ELISA kit (HerdCheck[®] Anti-Neospora; IDEXX Laboratories).

The ELISA kit uses sonicated lysate of tachyzoites as antigen and detects IgG. Assessment of the manufacturer's sensitivity and specificity of the test kit are 98.6% and 98.9% respectively (IDEXX, 2003). Test results were expressed as sample to positive control (S/P) ratios of optical density (OD). A test result was considered positive if it caused greater than 30% inhibition.

2.2.4 Data analysis

Questionnaire results were stored in a database (Microsoft Access® 1997, Microsoft Corporation, WA, USA) and further analyzed using Epi-Info 6.04 (Centre for Disease Control and Prevention, GA, USA,) and STATA® (STATA version 8; College Station, TX, USA,).

Individual and herd seroprevalence, estimated as the proportion of cattle and herds testing positive for *N. caninum*, respectively, were calculated. Then individual true prevalence and its 95% confidence intervals (95% CI) were estimated after correcting for test sensitivity and specificity (Dohoo et al., 2003). A herd was considered positive for *N. caninum* when at least 2 cows yielded a positive result in the ELISA test.

The identification of management variables associated with herd infection status (positive or negative) to *Neospora caninum* were assessed in two steps (Hosmer and Lemshow, 1989). First, univariable analyses comparing positive and negative herds to potential factors were performed. The outcome variable was *Neospora* herd status, while the independent variables were specific management variables selected from the questionnaire.

Normality was checked for continuous variables and logarithm transformations were used to achieve normality. If normality was not achieved, variable was categorized based on percentiles and analyzed as a categorical variable. Student's t-test was performed on continuous variables and the Chi-

square test (X^2) on the categorical variables. Variables that were significant at $P \leq 0.15$ in the univariate analyses were further included in an unconditional multivariable logistic regression model to determine main factors associated with herd seropositivity.

The model was built following a stepwise approach, with the variable with the smallest P -value entering first, followed by a forward selection and backward elimination for each variable using a likelihood ratio test at each step and based on P -value for entering ≤ 0.1 and a P -value for removing > 0.05 . Province and herd size were included in the final model as potential confounders regardless of their significance. Two-factor interactions between the main effects in the model were also tested in a similar fashion and goodness-of-fit was checked using the Hosmer and Lemeshow statistic (Hosmer and Lemeshow, 1989).

2.3 Results

2.3.1 Seroprevalence

Out of 4,700 farmers randomly selected, 280 (6%) initially responded to the mail out questionnaire, of which 179 (3.8%) eventually participated in the study. The final number of herds and cattle sampled in each province is shown in Table 1.

A total of 4,778 cows were tested from 179 herds, an average of 27 cows per herd. Forty-three percent (95% CI: 35.7 - 50.6) of herds in the study had at

least 2 seropositive cows. Apparent cow prevalence was 6.2% (95% CI: 5.47 - 6.83) while true cow prevalence was 5.2% (95% CI: 4.54 - 5.80). Within herd seroprevalence ranged from 25.0% to 75.9%. The distribution of results by province, herds and cows involved in the study are shown in Table 1.

2.3.2 Questionnaire results and statistical analysis

Of the 36 variables checked for possible association with *N. caninum* herd seropositivity only 8 were significantly associated (Table 3). “Pre-calving use of dry lots”, “separation of cow-calf pair from other cows after calving”, “use of standing water in summer”, “use of running water in winter”, “feeding heifers with manure handling equipment”, “abortion and stillbirths left for canids” and “number of sightings of wild canids per year” (categorized into three categories: less than 10 times per year, 11– 25 times per year, and greater than 26 times per year) were positively associated with herd serological status.

However, “washing boots between visits to livestock farms” was negatively associated with serological status. These 8 variables were included in a multivariable logistic regression model. Province and herd size were considered potential confounders and kept in the model regardless of significance.

Only 4 variables remained significant in the final model (Table 4). Herds that used dry lot as pre-calving area were 2.8 (95%CI: 1.25 – 6.22) times more likely to be seropositive for *Neospora caninum* when compare to herds that did not use dry lot housing. The odds of a herd being seropositive when exposed to natural

standing water as a source of water in summer was 3.2 (95%CI: 1.31 - 8.0) times higher compared to herds not exposed to the same type of water source.

High number of sightings of wild canids significantly increased the odds of a herd being seropositive. On average, when the number of sightings was more than 25 per year, the odds for a herd being seropositive increased 14.3 times (95%CI: 1.32 –153.7) compared to herds where farmers sighted coyotes and foxes less than 10 times per year.

The odds of being seropositive for herds leaving abortions and stillbirths for foxes and coyotes was 2.5 times (95% CI: 1.04 – 5.85) compared to herds that do not leave abortions and stillbirths for dogs and coyotes. The odds of being seropositive for herds from the Atlantic Canada (New Brunswick, Nova Scotia and Prince Edward Island) was 3.1 times greater (95%CI: 1.00 – 9.70) when compared to Ontario which was used as the province of reference. The model fit the data well (Hosmer and Lemeshow goodness-of-fit χ^2 statistic = 6.5; df = 8; $P=0.59$).

2.4 Discussion

From 4,700 Canadian farmers initially randomly selected, 280 (6%) showed willingness to participate in this study, but only 179 (3.8%) finally agreed to collaborate and their herds were sampled. The poor response rate in this study could be attributed to two major setbacks happening at the time the survey was

mailed, namely, a large-scale drought in Western Canada (2002 - 2003) and the emergence of bovine spongiform encephalopathy in Canada in 2003.

These two factors probably played a significant role in the reluctance of producers to participate in this survey, especially from Alberta and Saskatchewan, the two provinces with the highest beef cattle population and the lowest participation (Table 1). Voluntary participation in the study may have introduced a selection bias. Although this survey is one of the largest ever done in beef cattle in Canada, its results should not be considered representative of the Canadian beef cow-calf herds.

While research on the epidemiology of *N. caninum* in dairy cattle is extensive, fewer studies are published in beef cattle. Studies in beef cattle are more difficult to carry out because of the more extensive husbandry practices employed on beef cattle operations. Previous cross sectional studies in Canadian beef cattle have shown seropositivity to *N. caninum* ranging between 5.9% and 9% (Waldner et al., 2001 and 2005), which is similar to that found in this study, and would indicate a low seroprevalence of neosporosis in Canadian beef herds.

This seroprevalence is much lower than that found in a recent study carried out in several European countries (Bartels et al., 2006). Higher seroprevalence to *N. caninum* was found in Canada when investigating herds experiencing outbreaks of abortion or low reproductive performance (Waldner et al., 2005 and 2001). In

our study however, almost all farmers did not report high levels of abortion, which would support the low seroprevalence observed.

Different management systems employed for beef cattle may account for the presence of different risk factors associated with herd *N. caninum* status than those factors seen in dairy herd studies (McAllister et al., 2000). For example, the presence or the number of farm dogs had been associated with seroprevalence of *N. caninum* (Waldner et al., 2004; Hobson et al., 2005). We found no significant association between presence of dogs and beef cattle seropositivity to *N. caninum*.

In Canada, beef cows are mostly on the range, and potential contact with infectious oocysts could possibly be more likely at concentration points such as watering/feeding areas. In those areas, wild canids are the more likely definitive host expected, hence the possibility of beef cow-calf exposure to infectious oocysts from wild canids. Interestingly, in this study, seropositivity was associated with the sighting of wild canids (coyotes and foxes), a likely proxy of the probability that extensively managed beef cattle had of being exposed to the feces of definitive hosts of *N. caninum*.

The odds of being seropositive were 7.5 times (95% CI: 0.76 – 74.63) higher in those herds whose farmers saw coyotes and foxes between 11-25 times per year, as compared to herds where farmers sighted wild canids less than 10 times per year. These odds of having a seropositive herd increased even more when the number of sightings per year was ≥ 25 (OR=14.2; 95%CI: 1.3-153.7).

These results suggest the likely contamination of grazing pastures or water by *N. caninum* oocysts shed by foxes and coyotes. As the number of sightings of wild canids increased, the larger would be the likelihood of pasture or feed contamination.

The complete life cycle of *N. caninum* was recently revealed when it was shown that dogs fed with tissues of experimentally infected calves shed *N. caninum* oocysts (Dijkstra et al., 2001). It is expected that the same holds true for wild canids. In our study we found a significant relationship between herd seropositivity and farmers leaving abortions and stillbirths for dogs and wild canids. This association would support the idea that preventing the access of wild canids to fetuses and stillbirths may help to reduce pasture contamination and therefore lower seropositivity in the herd.

The use of natural standing water in summer was strongly associated with seropositivity (Table 3). Beef herds and wild canids may visit the same water source, thus the risk of water contamination or contamination of the surrounding areas by oocysts shed by wild canids will be possibly higher during this season.

Pre-calving use of dry lots was also found to be positively associated with herd seropositivity to *N. caninum*. The odds of a herd being seropositive were 2.8 times higher when pregnant cows were kept in dry lots instead of open pasture. Dry lots are usually associated with higher stocking densities, which may increase the probability of horizontal transmission in herds (Otranto et al., 2003).

Forty-three per cent of farms participating in this study had introduced at least one new animal into the breeding herd during the previous year. Although cow seropositivity had been positively associated with the purchase of new animals in a previous study (Mainar-Jaime et al., 1999), we did not find any such association in our study. Low individual seroprevalence observed may explain this lack of association, since the individual seroprevalence reported in the earlier study was 30.6%. Detailed individual cow information may help to describe the role of new additions in the epidemiology of neosporosis in beef cow-calf herds.

Italian researchers had suggested that the epidemiology of *N. caninum* infection in beef cattle might be different from dairy herds (Otranto et al., 2003; Rinaldi et al., 2005). The risk factors associated with seroprevalence in this study would suggest a similar conclusion. Wild canids seemed to play a significant role in the epidemiology of neosporosis in beef herds, the extent of which is not yet known. Beef herd managers might consider biosecurity practices such as preventing the access of wild canids to fetuses and stillbirths, and controlling water source contamination.

Limitations of our study were those inherent to any cross-sectional study, seroprevalence and potential risk factors were measured at a specific point in time. Thus, the associations found in this study are not necessarily causal. We can only hypothesize the role of the risk factors in *Neospora caninum* infection in beef cow-

calf. Thus, further studies on the epidemiology of this infection in beef cattle are warranted.

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Table 1. Distribution of herds, cows and province of origin in the study to determine the seroprevalence of *Neospora caninum* in beef cow-calf herds in Canada.

Province ^a	# Herds Sampled	Herd prevalence (%) ^b (95% CI)	# Cows sampled	Cow true prevalence (%) ^c (95% CI)
AB	29	75.9 (56.5 – 89.7)	833	9.0 (7.1 – 10.9)
BC	44	36.4 (22.4 – 52.2)	1196	3.8 (2.7 – 4.9)
ON	40	25.0 (12.7 – 41.2)	1037	3.3 (2.2 – 4.4)
SK	32	28.1 (13.8 – 46.8)	880	2.5 (1.5 – 3.5)
ATL	34	58.8 (40.7 – 75.4)	832	8.5 (6.6 – 10.4)
ALL	179	43 (35.7 – 51.0)	4778	5.2 (4.6 – 5.8)

^a AB = Alberta, BC = British Columbia, ON = Ontario, SK = Saskatchewan, ATL = Atlantic Canada, ALL = All provinces

^b A herd was considered positive if at least 2 cows were deemed seropositive

^c Based on a test

Table 2. List and description of variables in the study to determine the seroprevalence of *Neospora caninum* in beef cow-calf herds in Canada.

Sections	Variables
Farm and herd profile	Beef cow-calf operation: purebred, commercial, backgrounding or stocker.
	Breed composition: purebred-one breed, purebred-two breeds, British-type Crossbred cows, exotic-type crossbreds, crossbreds British and exotic.
Feeding management	Feedstuffs delivery: hay, green feed and straw -Bale, ground or manger
	Grain and silage – trough or ground
	Feedstuffs storage: hay, green feed and straw outdoor stack or covered stack
	Grain – hopper bottom bin or access bin. Silage – upright, pit silo or bags
	Mating method: natural mating, artificial insemination or mix
Breeding Management	Different groups of breeding cattle managed
	Proportion of breeding cows and heifers in the operation
	Total number of bulls in the operation used for breeding
	Breeding soundness evaluation

	Breeding at home before entering communal grazing
	Pregnancy check
	Heifers commingled with cows or managed separately in breeding season
	Communal grazing and contact with other livestock
Biosecurity practices	Maternity pens used as hospital pen in calving season
	Manure management and equipment contaminated.
	No. of new breeding animals brought onto the farm in the last 12 months
	Lending / borrowing / boarding of breeding cows, heifers or bulls
	Canids access to stored grain
	Attendance at shows and fairs and return to the farm
	Method of disposing abortion and stillbirths
	Dogs on farm, sighting of foxes, coyotes and roaming dogs
	Dry lot or pasture use as precalving area
	Area occupied by cowherd during calving season
	Abortions and stillbirths in previous year
	Calves born in each of previous 12months
	Calf mortality in last 12 months

Table 3. Univariable parameters associated with herd seropositivity in the study to determine seroprevalence of *Neospora caninum* in 179 beef cow-calf herds in Canada.

Variables	Univariable analysis		
	Herds (N=179)	% Positive	P -value
Provinces			
Ontario	40	25.0	<0.01
British Columbia	44	36.4	
Alberta	29	27.8	
Saskatchewan	32	28.1	
Atlantic Canada	34	58.9	
Pre-calving area			
Yes	75	32	0.01
No	104	51	
Cow-calf pairs separated from other cows after calving			
Yes	76	37	0.13
No	102	48	
Standing water source in summer			
Yes	104	53	<0.01
No	75	28	
Running water source in winter			
Yes	39	28	0.03
No	140	47	
Feeding heifers with manure handling equipment			
Yes	38	63	0.03
No	122	43	
Sighting coyotes and foxes			
<10 times/yr	87	34	0.03
11 - 25 times/yr	32	42	
> 25 times/yr	61	56	
Operators washing boots			
Yes	19	27	0.04
No	160	45	
Abortion and stillbirths left for dogs and coyotes			
Yes	53	53	0.01
No	126	39	

Table 4. Variables associated with herd seropositivity to *Neospora caninum* by multivariable logistic regression in 179 beef cow-calf herds in Canada.

Variables	Logistic regression parameters				
	<i>b</i>	<i>S.E. (b)</i>	<i>OR</i>	P	95% CI (OR)
Provinces					
Ontario	–	–	1	–	–
British Columbia	-0.19	0.67	0.7	0.78	0.22 – 3.05
Alberta	1.26	0.81	3.4	0.11	0.73 – 17.17
Saskatchewan	-0.77	0.72	0.4	0.28	0.11 – 1.89
Atlantic Canada	1.14	0.58	3.3	0.05	1.01 – 9.69
Breeding herd size					
≤46 head	–	–	1	–	–
>46 and ≤110 head	0.15	0.47	1.16	0.75	0.46 – 2.92
>110 head	-0.73	0.55	0.48	0.18	0.16 – 1.41
Pre-calving area					
Pasture	–	–	1	–	–
Dry Lot	1.02	0.41	2.8	0.01	1.24 – 6.22
Water source in summer					
No	–	–	1	–	–
Yes	1.17	0.46	3.2	0.01	1.31 – 8.01
Changing of boots					
No	–	–	1	–	–
Yes	-1.88	0.68	0.2	<0.01	0.04 – 0.58
Sighting coyotes and foxes					
<10 times/yr	–	–	1	–	–
>11 ≤ 25 times/yr	2.02	1.17	7.5	0.08	0.76 – 74.63
> 25 times/yr	2.66	1.21	14.2	0.03	1.32 – 153.74
Abortions and stillbirths left fordogs and coyotes					
No	–	–	1	–	–
Yes	0.90	0.44	2.5	0.04	1.04 – 5.85

3. The prevalence of antibodies to Bovine Leukemia Virus in Canadian beef cow-calf herds

3.1 Introduction

Enzootic bovine leukosis (EBL) is a lymphoproliferative disease of cattle caused by Bovine Leukemia Virus (BLV). The virus results in B-lymphocyte proliferation that is referred to as leukemia when the affected cells occur in the blood stream, lymphoma in the lymph node and lymphosarcoma in various vital organs (Willems et al., 2000).

Persistent lymphocytosis (PL) is the hallmark of BLV-induced leukosis. This results in a permanent increase in the number of B-lymphocytes in the peripheral blood (Meas et al., 2002). The importance of BLV as a production limiting disease lies in the potential loss of access to lucrative international markets and loss of consumer confidence (VanLeeuwen et al., 2001).

Clinical manifestations associated with BLV-infections in beef cattle include weight loss, agalactia, lymphadenopathy, anorexia, posterior paresis or paralysis, fever, exophthalmos, labored breathing, gastrointestinal obstruction, and myocardial abnormality; however, these symptoms only occur in about 3% of infected cattle.

Transmission of BLV occurs primarily via horizontal transmission of blood during routine livestock management practices. Iatrogenic transmission occurs during multiple uses of surgical blades for castration, multiple uses of tattoo

equipment and various dehorning tools (gougers, spoon, guillotine, and saws). Iatrogenic transmission also occurs from multi-use or multidose syringe, needles, and during the use of a common sleeve for rectal palpations (Di Giacomo et al., 1987; 1985, Lassauzet et al., 1990, Wilesmith et al., 1990; Brigtling and Radostits, 1983; Kohara et al., 2006). Biting flies (insect vectors – tabanids) have also been suggested to play a role in transmission (Manet et al., 1989).

One of the earliest reports of bovine leukemia virus (BLV) (Enzootic Bovine Leukosis, EBL) in Canada, found antibodies in 11% (79/703) of beef herds; the same study found antibodies in 40% (116/990) of dairy herds using agar gel immunodiffusion test (AGID). Overall herd prevalence estimates were 0.5% in beef herds and 9.3% in dairy cattle herds (Samagh and Keller, 1980).

A very extensive survey conducted in the United States supports the hypothesis that virus transmission occurs by contact transmission. BLV is common in large dairy herds and small beef herds, with animals typically housed more closely than in large beef herds and small dairy herds (NAHMS, 1997).

The objective of this study was to determine the seroprevalence of Enzootic Bovine Leukosis infection in beef cow-calf herds in selected provinces in Canada and identify risk factors associated with the herd seroprevalence.

3.2 Materials and Methods

3.2.1 Study population

The study population consisted of beef cow-calf herds from British Columbia, Alberta, Saskatchewan, Ontario, New Brunswick, Nova Scotia and Prince Edward Island. Surveys in Quebec and Manitoba were carried out separately (17). Promotional materials were initially sent to beef producers through the Canadian Beef Industry Associations in June 2002 and a reminder was sent before the commencement of the study. Confidentiality of responses to the questionnaire and of the results of individual serological results was assured and recruitment for the study was done in 2003 by random selection of 4,700 cow-calf producers in all participating provinces.

To estimate the number of cows to be tested in each herd the following assumptions were used: an average herd size of 45 cows, an expected average within-herd prevalence of 10%, an allowable error of 6%, and a level of confidence of 95%. Thirty cows per herds were thus required for testing (Martin et al., 1987).

3.2.2 Questionnaire survey

A comprehensive, 19-page mail-in questionnaire was designed to gather information on management, biosecurity and demographic factors. The questionnaire was divided into 6 sections: farm profile, calves and calving, feeding practices, veterinary procedures and vaccination, and farm biosecurity (a copy of

questionnaire is appended – Appendix 1). The information from the questionnaire used for the study is shown in Table 2.

3.2.3 Serology

During the fall of 2003, at the time of pregnancy diagnosis, blood samples were collected from randomly selected cows in each herd. The blood samples were collected by local veterinarians and animal health technicians. To ensure that cow selection was totally random, prior information on randomization methods had been sent to veterinarians and animal health technicians involved in blood collection.

Serum was harvested and stored at -20°C. The testing was done at The Canadian Food Inspection Agency Laboratory in Abbotsford, British Columbia using an enzyme linked immunosorbent assay (ELISA) (HerdCheck® BLV); IDEXX Laboratories Westbrook, Maine, USA).

The sensitivity and specificity of the test kit are 98.5%, and 99.9%, respectively (IDEXX 2003). An animal was considered to test positive for BLV antibodies, if the serum-to-positive (S/P) ratio was ≥ 0.05 , as suggested by the manufacturer (Simard et al., 2000). A herd was considered positive if one or more animal tested positive.

3.2.4 Data analysis

The questionnaire results were stored in a database (Microsoft Access® 2003, Microsoft Corporation, WA, USA) and further analyzed using Epi-Info 6.04

STATCALC (Centre for Disease Control and Prevention, GA, USA, 2003); and STATA® (STATA version 8; College Station, TX, USA, 2003). The individual and herd seroprevalence, estimated as the proportion of cattle and herds testing positive was calculated. The individual true prevalence and its 95% confidence intervals (95% CI) were estimated after correcting for test sensitivity and specificity. All information from Maritime Provinces (New Brunswick, Nova Scotia and Prince Edward Island) were grouped together and called Atlantic Canada for the purpose of data analysis.

The potential association between herd management variables and herd infection status (positive or negative) to Bovine Leukemia Virus was assessed (20). Univariable analyses using the Chi-square test and the t-test were used to compare each herd management variable that was a potential risk factor between positive and negative herds.

3.3 Results

3.3.1 Seroprevalence

A total of 4,778 cows were tested from 179 herds, an average of 27 cows per herd (range was 9-540). 1.2% (56/4778) of cows were seropositive (95% CI: 0 – 1.5%), while, 12.3 % (22/179) of herds had at least one seropositive cow present (95% CI: 7.5 – 17.1). After adjusting for Se and Sp, cow seroprevalence was estimated at 1.01% (95% CI: 0.73% – 1.29%) and herd seroprevalence was 12.4%

(95% CI: 7.57 – 17.23). The data for individual provinces, herds and cows involved in the study are shown in Table 1.

3.3.2 Summary of potential herd management risk factors

Of the 179 herds surveyed, 24% (43/179) of herd managers used surgical castration, 81.4% (35/43) of these herds practiced blade disinfection between animals. 14% (25/179) and 17.3% (31/179) dehorned cattle using gouger and saw methods respectively. 12.3% (22/174) of herd managers reported using new needles for each individual animal and most herd managers (53.6%, 96/179) reported that they did not change rectal sleeves between cows during rectal palpation.

The percentage of herds where calves received colostrum from their own dam was 44.7% (80/179), while 28% (50/179) of herds use colostrum from other cows, and 25.7% (46/179) from animals in the same herd. 5% (9/179) of herds used pooled colostrum, 21.2% (38/179) of herds used frozen colostrum, and 29.8% (40/179) used commercial substitute colostrum. Only 5% (9/179) of herds purchased a Holstein nurse cow for twins or orphaned calves.

6.1% (11/179) of herds reported calving location as pasture or open range; 31.8% (57/179) reported using small pastures; 26.3% (47/179) reported using corrals as a calving location and 31.8% (57/179) reported utilizing barns. Univariable analysis yielded no association between potential management factors and BLV herd seropositivity (Table 2).

3.4 Discussion

Seroprevalence of antibodies to Bovine Leukosis Virus in this study was low with an overall cow seroprevalence of 1.01% (95% CI: 0.73 – 1.3) and herd seroprevalence of 12.3% (95% CI: 7.5 – 17.1). No associations were established between the potential risk factors examined and herd level seropositivity.

Another study carried out in Manitoba sampled and tested 1,425 beef cows and 1,204 dairy cows from 49 beef and 40 dairy herds respectively (VanLeeuwen et al., 2006). This study reported cow seroprevalence of 10.3% (95% CI: 2.5 -18) and herd prevalence of 47.9% (95% CI: 28 - 68) in beef cattle.

As in the current study, no management factor was found to be associated with seropositivity in the Manitoba study. The same study reported 60.8% (95% CI: 51.8 – 69.9%) cow seroprevalence and 97.4% (95% CI: 93.5% -100%) herd seroprevalence in dairy cattle. Herd level seropositivity was established when at least 1 animal tested positive in a herd (VanLeeuwen et al., 2006).

Another study of dairy herds in Atlantic Canada reported cow seroprevalence of 20.8% (95% CI: 15.8 - 27.0), and herd seroprevalence of 70.0% (95% CI: 60.3 - 79.7), (VanLeeuwen et al., 2001). Similarly, a study carried out in dairy herds in Saskatchewan found 37.4% (95% CI: 28.8 - 46.0) cow seroprevalence and 89.1% (95% CI: 80.8 - 97.4) herd seroprevalence in 1,530 dairy cows from 51 herds (VanLeeuwen et al., 2005).

These studies show a higher cow and herd seroprevalence for antibodies to BLV in dairy cattle compared to beef cattle. An earlier study suggested contact transmission as the reason for high dairy cow and herd seroprevalence due to close housing (NAHMS, 1997).

Significant differences in cow and herd level seropositivity in dairy cows compared to beef cows was observed in Saskatchewan compared to Maritime Provinces (VanLeeuwen et al., 2005). More dairy cows (37%) and herds (89%) tested positive in Saskatchewan compared to dairy cows (21%) and herds (70%) from Maritime Provinces. This study suggested different virus transmission methods and levels of exposure as a reason for variation in level of seroprevalence among the provinces.

While the reasons for the disparity are unknown, the conclusion of the current study is that BLV seroprevalence is low in beef cattle in Canada. No associations existed between potential risk factors and seropositivity to BLV in this study, likely because the number of herds testing positive to BLV were too few to find any associations.

3.5 References

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Table 1. Seropositivity to Bovine Leukosis Virus in beef cow-calf herds in Canada showing provinces, herds and cows sampled with herd and cow level seropositivity.

^a Provinces	# Herds Sampled	^b Positive Herds	# Cows sampled	^c Positive cows
BC	44	1	1196	1
AB	29	1	833	1
SK	32	4	880	32
ON	40	8	1037	29
ATL	34	8	832	34
ALL	179	22	4778	56

^a AB = Alberta, BC = British Columbia, ON = Ontario, SK = Saskatchewan, ATL = Atlantic Canada, ALL = All provinces

^b A herd was considered positive if 1 cow tested seropositive

^c Based on a test sensitivity of 98.5% and specificity of 99.9%

Table 2. List and description of variables examined in a study of the seroprevalence of antibodies to Bovine Leukosis Virus in beef cow-calf herds in Canada.

Section	Variable
Farm and herd profile	- Beef cow-calf operation: purebred, commercial, backgrounding or stocker.
	- Breed composition: purebred-one breed, purebred-two breeds, British-type
	- Crossbred cows, Exotic-type crossbreds, Crossbreds British and exotic.
	- Primary or secondary business and source of income.
	- Time devoted to the operation and age of person making day-to day decisions.
Animal Processing	- Dehorning methods: gougers, saws or guillotine, electric dehorner, caustic paste
	- Castration methods: Surgical, rubber bands, clamps or rubber rings
	- New needle used for every injection
	- Rectal examination: changing of rectal gloves between animals
Calving management	- Density in maternity pen
	- Colostrum: from other cows and pooled
	- Use of Holstein cow to nurse orphan calf.
	- Density in pre-calving area.

4. Biosecurity practices in beef cattle literature review

4.1 Introduction

Biosecurity practices are activities undertaken to prevent the introduction and spread of disease agents into an area. The area of interest may be at the farm level, provincial or state level, regional or national level. At the farm level, preventing the introduction of infectious agent, reducing transmission within the herd, and preventing diseases from leaving the farm are different aspects of disease control (Anderson, 1998, Radostits, 2001).

On-farm biosecurity practices employ a variety of approaches to manage disease determinants by identifying and controlling for management practices having the potential of introducing new pathogens.

4.2 Addition of new animals

The addition of calves, cows or bulls into a production unit from other farms or producers is a potential source for new pathogens in the herd. For example, the addition of new calves can potentially introduce BVDV into a herd by purchasing calves persistently infected with BVD virus (Houe, 1995).

Calves purchased from sale yards was described as a risk factor for enteric and respiratory diseases especially when calves have been commingled from several sources of origin and exposed to a wider variety of pathogens (Fulton et al., 2005; Callan and Garry, 2002). Multiple disease agents cause enteric infections

in neonatal beef calves; as such, the management practices adopted have to minimize the risk of enteric infection. Sound management practices may protect from enteric and other infections in neonatal calves (Moon et al., 1978).

4.3 Exposure to other herds

Venereal diseases such as trichomoniasis and campylobacteriosis can enter a herd through the addition of bulls or breeding females. Commingling of herds in communal grazing situations is a significant risk factor for venereal diseases. A prevalence study of 57 randomly selected beef cow-calf herds found 15.8% (9) herds with at least one infected bull. Seven hundred and twenty nine (729) bulls sampled had 4.1% (30) culture positive for *Tritrichomonas fetus* (BonDurant et al., 1990). Another prevalence study found *T. fetus* prevalence of 11.9% among bulls in a large multi-unit ranch with bull group prevalence ranging from 0% to 39% (Rae et al., 1999).

4.4 Feeds and feeding

Contamination of feeding and drinking areas was associated with outbreaks of neosporosis in beef cattle (Dijkstra et al., 2002). Shared pasture or communal grazing, contact at fairs or exhibitions, lending cow or bulls and borrowing cow or bulls may have the risk for introducing new disease into a herd. Contact between

livestock, domestic canids and wild canids was a risk factor for *Neospora caninum* (Gondim et al., 2004; McAllister et al., 1996)

4.5 Manure management

Salmonella typhimurium can contaminate the soil after incorporation of contaminated bovine manure into the soil (Natvig et al., 2002). Pasture contamination may occur by spreading manure on surface soil and carrying the potential risk factor for infection with *Salmonella typhimurium* during grazing.

Mycobacterium avium paratuberculosis (MAP) can persist for 9 to 55 weeks in bovine manure and on pasture (Whittington et al., 2004). Sharing of manure-contaminated equipment between livestock farms may have the potential for transmitting infections between the farms sharing the contaminated equipment. *Salmonella typhimurium*, a potential zoonotic agent, survived in the environment from fecal material in dry or wet substances for 4-5yrs (Plym-Forshell and Eksebo, 1996).

4.6 Vaccination

Traditionally, the use of vaccination in cattle populations has prevented the occurrence of infectious diseases and remains a powerful biosecurity tool. However, the sole use of vaccination for the prevention of infectious disease may not be sufficient in preventing the occurrence of infectious disease in beef cattle

herds. Calves vaccinated with bovine viral diarrhea vaccine are at risk of infection in the face of constant challenge with Bovine Viral Diarrhea Virus shed by a persistently infected (PI) calf in the herd. While vaccination may help in offering protection to the calves, testing will identify the PI calf for removal.

Experimentally, transmission of Bovine Viral Diarrhea Virus has occurred via contaminated vaccines in dairy cattle when using needles for vaccination (Barkema et al., 2001; Falcone et al., 2003). Poor hygienic practices and environmental contamination was suggested to play a role in transmission of BVDV (Niskanen and Lindberg, 2003; Lindberg et al., 2004). Multiple uses of needles during vaccination and the same tattoo instrument without disinfection has been associated with BLV transmission in dairy replacement heifers (Wilesmith 1979; Brightling and Radostits, 1983; Lassauzet et al., 1990).

4.7 Other biosecurity practices

Risk of infectious disease transmission may occur when using the same rectal gloves between animals during rectal palpation (Kohara et al., 2006). The use of hospital pens as a maternity pen in beef cowherds may predispose immunologically naïve newborns calves to infections. Animal processing practices promoting cross-contamination of body fluids between animals may have the potential for transmitting diseases. Gouge dehorning method transmitted BLV infection in dairy replacement heifers (DiGiacomo et al., 1985 and 1987).

Other practices promoting cross-contamination of body fluids between infected and non-infected animals includes surgical castration with a single scalpel blade without disinfection, multiple uses of ear-tag /notching equipment without disinfection and the use of same needle for injecting vaccines.

Hospitalization of sick animals and returning them to the farm of origin is a potential biosecurity risk due to nosocomial infections. Manure contaminated clothing and boots, vehicle tires contaminated with manure, and visits to different farms by animal health workers may have a potential for transmission of disease (Dargatz et al., 2002).

In establishing an effective biosecurity program, considerations should include the epidemiological triad of host, agent (reservoir /vector) and the environment. In addition, pathogen characteristics, modes of transmission (direct and indirect), environmental survivability, incubation period and period of communicability are of great concerns (Barrington et al., 2002). Lapses in biosecurity practices may result in the introduction of disease into a new herd resulting in increased morbidity / mortality, decreased productivity, decreased reproductive efficiency and the loss of marketing options (Anderson et al., 1998; Dargatz et al., 2002)

Traditionally, on-farm vaccination has been used as a disease control tool preventing untoward effects of pathogens in production animal population. Vaccination with all its merits has proven to be insufficient in preventing the

effects of infectious disease at farm level (Dargatz et al., 2002). As such, consideration of more inclusive approach of all farm management practices may be useful in disease prevention.

Farm management practices have a potential to introduce disease risks. Identification, evaluation, and addressing the risks are essential to a sound biosecurity program. Recent emphasis on farm level biosecurity in production animal medicine may be due to emergence of infectious disease problems with zoonotic potential in farm animal populations (Dargatz et al., 2002).

The objective of this study was to evaluate biosecurity practices of beef cow-calf herds in Canada by identifying practices that may increase introduction and transmission of pathogens in beef cow-calf herds.

4.8 Materials and Methods

4.8.1 Study population

The study population consisted of beef cow-calf herds from British Columbia, Alberta, Saskatchewan, Ontario, New Brunswick, Nova Scotia and Prince Edward Island. Surveys in Quebec and Manitoba were carried out independently (17). Promotional materials were initially sent to beef producers through the Canadian beef industry associations in June 2002, a reminder was later sent before the commencement of the study. Confidentiality of response to questionnaire and serological analysis were assured and recruitment for the study

was done in 2003 by random sampling of 4,700 cow-calf producers in all participating provinces.

4.8.2 Producer recruitment and questionnaire survey

Producer recruitment for the study was done in 2003 by random sampling of 4,700 cow-calf producers in all participating provinces and promotional materials were mailed to all the identified producers.

A comprehensive, 19-page mail-in questionnaire was designed to gather information on management, biosecurity and demographic factors. The questionnaire was completed by the herd manager and confirmed by an extension worker. The questionnaire was divided into 6 different sections: farm profile, calves and calving, feeding practices, veterinary procedures and vaccination, and farm biosecurity (copy of questionnaire is appended – Appendix 1).

4.8.3 Data analysis

Biosecurity-related management practices selected from all sections of the questionnaire with similar biosecurity related variables were grouped together and analyzed for the prevalence of the management factor. Biosecurity practices were evaluated by province, herd size and region (eastern or western Canada).

4.9 Results

4.9.1 General population characteristics

A total of 179 herds participated in the study representing approximately 15,738 cows and 34,359 beef cattle (See Table 1 for distribution of herd sizes and provinces). Herds enrolled in the study included both purebred and crossbred herds. Crossbred herds represented 62.0% (111/179), while purebred herds represented 38.0% of total herds enrolled (68/179).

4.9.2 Addition of new animals

Seventy three percent (73.7%, 132/179) of herds added new animals to their herds within the 12 months prior to the survey. Unweaned beef calves (13%, 23/179), weaned beef calves (18.4%, 33/179), bred beef heifers (17.3%, 31/179) bred beef cows (23.3%, 43/ 179) and 44% (79/179) weaned bulls were added to the herd 12 months prior to the survey (2002 and 2003) (Table 2).

Of those herds that reported purchasing animals from outside sources, 58% (104/160) of herds reported buying animals directly from other producers, 26.8% (48/160) reported buying animals from auction markets, and 4.5% (8/160) reported buying animals from private dealers. 5 years prior to the survey (in the period between 1997 and 2001), new additions to the herds were 89.4% (160/179) while 10.6 % (19/179) of herds were reported as closed herds. 16.2% (29/179) of herds reported adding an unweaned Holstein calf, 18.4% (33/179) reported adding

an unweaned beef calf and 5% (9/179) reported adding a Holstein nurse cow in the period between 1997 and 2001 (Table 2).

4.9.3 Exposure to other beef herds

In the 5 years prior to the study, exposure to feedlot cattle was reported at a prevalence of 10.6% (19/179). 60% (107/179) of herds had fence-line contact with other beef cows herds while on pasture. Lending and borrowing of cows and bulls from other herds was reported at a prevalence of 25.7% (46/179) and 19% (34/179) respectively. 29.6% (53/179) of herds reported using communal pasture in the 5 years prior to the study. The prevalence of exposure of beef cow-calf herds to other beef herds in the 5 years prior to the study is shown in Table 3.

Twenty four percent (43/179) of herds reported using communal pasture 12 months prior to the survey (Table 3) and 28% (12/43) of these herds reported using more than one communal pasture. Large herds (≥ 111) were more likely to use communal pasture compared to smaller herds (≤ 46) ($P < 0.01$). Herds from western Canada were more likely to use communal pasture compared to herds from eastern Canada ($P < 0.01$). Twenty three percent (41/179) of herds reported having animals that attended fairs where they were exposed to beef cattle from other herds.

4.9.4 Exposure to other species

Herds were exposed to other species of animals that included pigs, goats, sheep, chickens/poultry litter, bison, llamas/alpacas, horses/other equidae and

captive elk/deer (Table 4 shows summary of data on exposure to other species of animals). 85.4% (153/179) of herds had contact with farm dogs.

Exposure of beef cow-calf herds to coyotes / wolves, foxes and roaming dogs occurred in this study (Table 5). Rodent infestation on farms was categorized and reported as low, medium and high, with the following percentage of farms in each category: low (73.7%, 132/179), medium (23.5%, 42/179) and high (2.8%, 5/179).

4.9.5 Manure management

The use of manure equipment for feeding heifers occurred in 31.3% (56/179) of herds. Of herds using manure equipment for feeding heifers, 78.6% (44/56) used the same equipment occasionally while 21.4% (12/56) used the same equipment regularly. In cows, 38.5% (69/179) of herds reported using manure equipment to feed cows. Of herds using manure equipment for feeding cows, 81.2% (56/69) used the same equipment occasionally while 23.2 % (16/69) used the same equipment regularly. 21.2% (38/179) of herds reported spreading manure on land used as pasture for replacement heifers as shown in Table 6.

Fifty-two percent (93/179) of herds borrowed equipment contaminated with manure from other cow-calf operations. Thirty-three percent (59/179) of herds reported disposing of manure by spreading on surface soil without soil incorporation (Table 7).

4.9.6 Feeds and feeding

Feedstuffs administered on the ground were hay (50.3%, 43 – 57.5), green feed / baled cereal (38.6%, 31.7 – 45.9), straw (45.3%, 38.1 – 52.6), and grain (16.2%, 11.5 – 22.3). Domestic and wild canids had access to stored grain in 19% (34/179) of herds. The odds of wildlife gaining access to stored grain is twice as high (OR=2.37, $P<0.02$) in western Canada compared to eastern Canada. See Table 8 for summary of feedstuffs administered on the ground and the access of canids to stored grain.

Purebred herds were less likely to be fed on the ground compared to cross-bred herds ($P<0.03$). Herds from western Canada administered more feedstuffs on the ground compared to herds from eastern Canada ($P<0.00$). Large herds were more likely to store feedstuffs outdoors compared to small herds ($P<0.01$). Herds from western Canada were more likely to store their feedstuffs outside compared to eastern Canada ($P<0.00$).

4.9.7 Breeding management

Herds divisions into breeding groups were - one group (32.4% 58/179), two groups (30.7, 55/179), three groups (17.3%, 31/179), four groups (8.4%, 15/179), and over five groups (6.7%, 12/179). Eighty three percent (148/178) of herds have a defined breeding season, 6.7% (12/179) of herds have an undefined breeding season, while 10.6% (19/179) of herds have different breeding seasons.

Sixty three percent (113/179) of herds used natural mating, 3.9% (7/179) of herds used artificial insemination, and 33% (53/179) of herds used a mixture of natural mating and artificial insemination. 15.6% (28/179) of herds reported breeding cows at home prior to entering communal pasture.

Thirty three percent (53/179) of herds reported performing breeding soundness examinations in breeding bulls and 9.5% (17/179) of herds performed trichomonas testing on breeding bulls. See Table 9 for summary of data on trichomonas testing.

60.1% (109/179) of herds reported pregnancy testing via rectal examination by a veterinarian. 4.7%(8/179) of herds performed pregnancy testing via rectal examination by a non-veterinarian, and 7.8% (14/179) of herds reported pregnancy testing by visual examination.

Sixty percent (107/179) of herds used the same area for calving and winter-feeding. Herds from western Canada were more likely to use the same area for calving and winter feeding when compared to herds from eastern Canada ($P<0.00$). Forty-one percent (73/179) of herds used hospital pens as maternity pens during calving season. The odds of using maternity pen as hospital pen was twice as high in western Canada when compared to the eastern Canada ($OR=2.0$, $P=0.04$).

Fifty-seven percent (102/179) of herds separated cow-calf pairs from pregnant cows after calving. Sixty percent (107/179) of herds used the same area

for calving and winter-feeding. Fifty seven percent (102/179) of herds used dry lot as pre-calving area.

4.9.8 Other biosecurity practices

35.8% (64/179) of herds transported animals to veterinary clinics for treatment. In nine percent (16/179) of herds, visitors or outside employees changed their boots, and in 18% (32/179), visitors washed their boots. See Table 9 for summary of data on veterinary visits, changing of boots, changing of clothes and washing of boots by visitors / outside employees.

Forty-three percent (76/179) of operators spend time in other beef cow-calf operations, during which 10.6% (19/179) changed their boots, 8.9% (18/179) changed their clothes and 16.8% (30/179) washed their boots. See Table 10 for summary of data on changing boots, changing clothes and washing boots by the operator when visiting other beef cow-calf farms.

Eighty two percent (146/179) of herds dehorned cattle, of which 74% (109/148) used non-bloodless method. Of herds using non-bloodless dehorning method, 28.4% (31/109) disinfected dehorning equipment between animals. 73.7% (132/179) of herds reported castrating animals, of which 32.6% (43/132) used surgical castration method. Of herds using surgical castration method, 81.4% (35/43) disinfected surgical equipment between animals. 12.2% (22/179) of herds disinfected or used new needles between animals when injecting drugs or

vaccines. Thirty five percent (63/179) of herds changed sleeves between animals when performing rectal examinations.

Methods employed in the disposal of abortions and stillbirths included burying, disposal in landfill, rendered, eaten by cows/heifers and left for dogs/coyotes. 22.3% (40/179) of herds buried abortions while 23.5% (42/179) of herds buried stillbirths. Herds landfilling abortions and stillbirths were 6.7% (12/179). 8.9% (16/179) of herds rendered abortions while 12.3% (22/179) of herds rendered stillbirths. 29.6% (53/179) of herds left abortions for dogs and coyotes while 27.4% (49/179) of herds left stillbirths for dogs and coyotes, and 2.2% (5/179) of herds left abortions for cows to eat.

4.9.9 Bovine Viral Diarrhea (BVD) Virus Vaccination

20.1% (36/179) of herds vaccinated calves for BVD virus, of which 6.7% (12/179) used modified live vaccine and 13.4% (24/179) used killed vaccine. 51.4% (92/179) of herds vaccinated heifers and steers at weaning with BVD vaccine. Of heifers and steers vaccinated, 18% (37/179) were vaccinated with modified live vaccine, while 33.5 (60/179) were vaccinated with killed vaccine.

Sixty percent (107/179) of herds vaccinated replacement heifers (yearlings) with BVD virus vaccine. Of herds vaccinating replacement heifers for BVD virus, 25.1% (45/179) used modified live virus vaccine while 34.6% (62/179) vaccinated with killed vaccine. BVD virus vaccine in replacement heifers was administered

prior to breeding (41.3%, 74/179), during pregnancy check (13.4%, 24/179) and at other times (5%, 9/179).

Herds vaccinating breeding cows for BVD virus were 63.1% (113/179), of which 29.1% (52/179) used modified live vaccine and 34.1% (61/179) used killed vaccine. BVD virus vaccine in breeding cows was administered prior to breeding (29.6%, 53/179), during pregnancy check (28%, 50/179) and during other times (5.6%, 10/179). See Table 11, for summary of data on BVD virus vaccination.

5. Discussion

Lapses in management practices observed in this study may constitute biosecurity risks to beef cow-calf herd operations. Addition of new animals to the herds occurred at an overall prevalence of 73.7% (95% CI: 67.3 – 80.2), with 26.8% (95% CI: 20.3 – 33.1) of herds sourcing new additions from auction markets. Compared with the US, 47.8% (SE \pm 12.2) of herds added new animals with 34.8% (SE \pm 2.1) of herds sourcing new additions from auctions (USDA 2009, Part II Beef 2007-2008, #N512.0209).

In western Canada, the auction system accounts for over 80% of beef cattle sales, over 70% of Canadian cow-calf herds are located in the same area. Beef calves and cows make at least 2 trips to auction pens in their lifetime, as juveniles and later as adult cattle and may mingle with other cattle.

Thirteen percent (95% CI: 8 – 17.8) of herds added unweaned beef calves compared with 8.0% (SE \pm 2.7) of herds in the US that added unweaned beef calves (USDA 2009, Part II Beef, 2007-2008, #N512.0209).

Addition of Holstein nurse cows and Holstein calves occurred at a prevalence of 5% (95% CI: 1.8 – 8.2) and 16.2 (95% CI: 8 – 17.8) of herds in this study. This may be compared to herds in the US where the addition of Holstein nurse cows and Holstein calves occurred at a prevalence of 1.2% (SE \pm 0.9) and 0.3% (SE \pm 0.2) (USDA 2009, Part II Beef 2007-2008, #N512.0209). Addition of Holstein cows of unknown disease status and without pre-screening tests may carry the risk of introducing Johne's (*M. paratuberculosis*) disease.

Since sourcing of high proportion of new additions (73.7%), especially calves from various auctions (26.8%) without pre-purchase screening presents risks of exposure to respiratory pathogens (O'Connor et al., 2005; Callan and Garry, 2002), we hypothesize that calves from herds participating in this study may be at a risk of transmission of respiratory infections.

Exposure to other beef herds on communal / shared pasture occurred at a prevalence of 28% (95% CI: 14.5 – 41.3), compared with 7.8% (SE \pm 0.8) of herds in the US. A similarity observed between herds in this study and herds in the US was an increase in the odds of using communal pasture that increased with herd size (USDA 2008, Part I Beef 2007-2008, #N512.1008).

Exposure to animals from other beef herds at shows / fairs was prevalent at 23% (95% CI: 16.8 – 29.1) compared with 5.4% (SE \pm 0.6) of herds in the US. Fence-line contact with other beef operations was prevalent at 60% (95% CI: 52.6 – 67) compared with 96.3% of herds in the US. In Canada, 85.4% (95% CI: 80 – 90.2) of herds had contact with farm dogs compared with 68% of herds in the US (USDA 2009, Part II Beef 2007-2008, #N512.0209).

Herds performing breeding soundness examinations in bulls were 33% (95% CI: 23 – 36.3) of herds, compared with 26.8% (SE \pm 1.2) of herds in the US. *Trichomonas* testing on breeding bulls was prevalent at 9.5% (95% CI: 5.2 – 13.8) of herds compared with 9.8% (SE \pm 0.8) of herds in the US (USDA 2008, Part III Beef 2007-2008, #N518.0509).

There was a low proportion (15.6%, 95% CI: 10.6 – 21.4) of cows breeding before entering communal or shared pastures where mating occurs with high proportion (24%, 95% CI: 17.7 – 30.1) of bulls from other herds. Considering the low proportion (9.5%, 95% CI: 5.2 – 13.8) of herds testing bulls for *T. fetus*, there are risks of exposure of cows to venereal diseases carried by the bulls.

With high proportion (25%, 95% CI: 19.5 – 32.1) of herds lending cows and bulls, and returning them to herds of origin, we hypothesize that herds participating in this study may be at a risk of transmission of venereal disease, including *Trichomonas* and *Campylobacter*. Communal grazing was a risk for infection with *Trichomonas fetus* in a US study (Gay et al., 1996).

The use of manure handling equipment for feeding heifers and cows was prevalent at 31.3% (95% CI: 24.5 – 38.1) and 38.5% (95% CI: 31.4 – 45.7), compared with 15% (SE \pm 1.2) of herds in the US. Borrowing / sharing of manure contaminated equipment was prevalent at 52% (95% CI: 44.6 – 59.3) compared with 17.3 (SE \pm 3.1) of herds in the US. Feeding heifers with manure handling equipment was positively associated with herd serological status for *Neospora caninum*.

Manure disposal by surface spreading was prevalent at 33% (95% CI: 26.9 – 40) compared with 29.5% (SE \pm 1.1) of herds in the US (USDA 2008, Part I Beef 2007-2008, #N512.1008). Borrowing / sharing manure contaminated equipment has the potential for transmitting diseases between farms, especially when the same equipment is shared between many farms.

Dogs / cats / wildlife had access to stored grain in 19% (95% CI: 13.2 – 24.7) of herds, compared with 35.8% (SE \pm 0.8) of herds in the US (USDA 2009, Part II Beef 2007-2008, #N512.0209). Dog / cats / wild canids having access to stored grain was a risk factor for bovine neosporosis. Fecal contamination of uncovered feedstuffs stored outside and administration of the same feedstuffs may allow for transmission of pathogens, including *Neospora caninum* (Sanderson et al, 2000).

High number of sightings of wild canids may increase the odds of disease transmission from wild canids to beef cow-calf herds. High number of sightings of

wild canids significantly increased the odds of a herd being seropositive for *N. caninum*. On average, when the number of sightings of coyotes and foxes was more than 25 per year, the odds for a herd being seropositive increased 14.3 times (95% CI= 1.32 –153.7) compared to herds where farmers sighted coyotes and foxes less than 10 times per year.

Forty-one percent (95% CI: 33.6 – 48) of herds used the calving area as a sick pen compared with 28.7% (SE \pm 2.1) of herds in the US. Fifty-seven percent (95% CI: 50 – 64.2) of herds separated cow-calf pairs from pregnant cows after calving compared with 14% (SE \pm 0.8) of herds in the US (USDA 2009, Part II Beef 2007-2008, #N512.0209).

Non-separation of cow-calf pair from other pregnant cows after calving could expose naïve newborn calves to a higher pathogen load and increase the possibility of neonatal infection that may occur at calving and during the post parturient period (Lindberg et al., 2004). Separation of cow-calf pair from other cows after calving was positively associated with herd serological status for *Neospora caninum*.

Fifty seven percent (95% CI: 50 – 64.2) of herds used dry lots as pre-calving area, the use of dry lots as a pre-calving area was positively associated with herd serological status to *Neospora caninum*. Dry lots are usually associated with higher stocking densities, which may increase the probability of horizontal transmission in herds (Otranto et al., 2003).

The use of the same area for calving and winter-feeding was prevalent in 60% (95% CI: 52.6 – 67) of herds. Use of the calving area as a sick pen may expose neonatal calves to environmental pathogens before passive transfer of immunity via colostrum, including *Salmonella typhimurium* and *Escherichia coli* (Whittington et al., 2004; Plym-Forshell and Eksebo, 1996).

Disinfection of dehorning equipment between animals was prevalent at 28.4% (95% CI: 20 – 37) compared with 85.7% (SE \pm 7.4) of herds in the US. Cross-contamination of dehorning equipment resulted in transmission of pathogens (DiGiacomo et al., 1987; Lassauzet et al., 1990).

Potential risk exists for transmission of pathogens with 81.4% (95% CI: 68 – 93) of herds disinfecting blades during castration, 12.3% (95% CI: 7.5 – 17.1) of herds using new needles for injection and 35.2% (95% CI: 28.2 – 44.2) of herds changing sleeves between cows during rectal palpation (Wilesmith et al., 1983; Kohara et al., 2006, Niskanen and Lindberg, 2003)

Transporting animals to veterinary clinics for treatment and returning them to the herd of origin occurred in 35.8% (95% CI: 28.7 – 42.7) of herds. This practice could compromise herd biosecurity as it carries the risk of transmitting nosocomial infection to the herd of origin.

The perception of risk varied between visitors / outside employees and the operator, as revealed in the practice of changing and washing of boots. Visitors / outside employees changed and washed boots at the prevalence of 9% (95% CI:

4.8 – 13.1) and 18% (95% CI: 12.3 – 23.5). Comparatively, operators changed and washed boots at the prevalence of 10.6% (95% CI: 6.1 – 15.1) and 16.8% (95% CI: 11.3 – 22.2) when visiting other beef cow-calf operations. Washing boots between visits to livestock farms was negatively associated with serological status for *Neospora caninum* in this study.

29.6% (95% CI: 23 – 36.3) of herds left abortions for dogs and coyotes while 27.4% (95% CI: 20.8 – 34) of herds left stillbirths for dogs and coyotes. Leaving abortion and stillbirths for dogs and coyotes was a risk factor for *N. caninum*. The odds of being seropositive for herds leaving abortions and stillbirths for foxes and coyotes was 2.5 times (95% CI=1.04 – 5.85) compared to herds that did not leave abortions and stillbirths for dogs and coyotes.

The proportions of herds vaccinating cows and replacement heifers for BVD virus were less than two-thirds of total herds enrolled in the study, with less than half of vaccinating cowherds using a modified live vaccine. About half of the herds vaccinated the cowherds for BVD virus prior to breeding while the other half vaccinated during pregnancy check.

Although BVD virus vaccination may compensate for exposure to BVDV in a cowherd by mitigating the risk of fetal infection, the timing of vaccination is very essential. BVD virus vaccination administered at pregnancy check may not protect the fetus against BVDV infection. There is likelihood for BVDV infection during gestation resulting in the birth of an immunotolerant fetus with persistently

infection (PI). This may occur if the calf was infected in-utero before 125 days of gestation.

PI calves are sources of BVDV infection in the herd by shedding large amounts of the virus. BVD virus vaccination aimed at reducing transmission is one of the multi-dimensional approaches to reducing BVDV transmission in the cowherd (Grooms et al., 2009). Since less than two-thirds cowherds vaccinated for BVDV, a larger proportion of calves vaccinated with a modified live vaccine for BVDV before weaning could compensate for low cow herd vaccination. However, BVDV vaccination occurred in 20% of calves before weaning.

Beef cow-calf herds in this study were involved in management practices constituting risks and which could introduce diseases into the herd. The perception of what constitute risks and the approach to mitigating the risks varies among cow-calf operators in this study. Producer education in identifying risks and lapses in biosecurity needs emphasis. The role of veterinarians in producer education and awareness of minimal biosecurity standards in beef cow-calf is important to mitigating risks and the success of biosecurity program in beef cow-calf.

Limitations of our study were those inherent in any cross-sectional study. Cause and effect inference cannot be made with certainty; we can only hypothesize the role of lapses in biosecurity practices in transmission of infection in beef cow-calf herds in Canada. Thus, further studies needs be carried out on biosecurity practices in beef cow-calf in Canada. Selection bias in producer

recruitment and voluntary participation may have contributed to under-estimation of prevalence of biosecurity practices in this study, especially from Alberta and Saskatchewan, the two provinces with the highest beef cattle population and the lowest participation.

The role of the veterinarian is essential in educating producers on what constitute risky practices and how to mitigate such risks. Approach to mitigating risks may not be the same for all cow-calf herds; but tailored to the need of each production unit.

Initial risk assessment will identify what constitutes risky management practices, after which sound mitigation measure are designed to address such risks. On-farm biosecurity practices needs approach within the framework of risk assessment and periodic review for effectiveness (Ortiz-Pelaez and Pfeiffer, 2008).

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Table 1: Distribution of herds, sizes and provinces in the study describing biosecurity practices of beef cow-calf herds in Canada.

Province ^a	# Herds	# Cows
BC	44	5230
AB	29	3996
SK	32	3185
ON	40	1888
ATL	34	1439
ALL	179	15738

^a BC = British Columbia, AB = Alberta, SK = Saskatchewan, ON = Ontario,

ATL = Atlantic Canada, ALL = All provinces

Table 2: Addition of unweaned beef calves, weaned beef heifers, bred beef heifers, and bred beef cows 12 months before the study and the addition of Holstein nurse cow, unweaned Holstein calves and unweaned beef calves 5 years before the study describing biosecurity practices of beef cow-calf herds in Canada.

Province ^a / Herds	% adding unweaned beef calves ^b	% adding weaned beef heifers ^b	% adding bred beef heifers ^b	% adding bred beef cows ^b	% of herds adding a Holstein nurse ^c cow	% of herds adding an unweaned Holstein calf ^c	% herds adding unweaned beef calf ^c
BC (44)	9 (0.6 - 17.6)	16 (5.1 - 26.7)	9 (0.6 - 17.6)	25 (12.2 - 37.8)	4.6 (1.6 - 10.7)	9 (0.6 - 17.9)	25 (12.2 - 37.8)
AB (29)	13.8 (1.24 - 26.3)	17 (3.5 - 31)	10.3 (0.7 - 21.4)	13.8 (1.2 - 26.3)	13.8 (1.2 - 26.3)	13.8 (1.2 - 26.3)	27.5 (13 - 44)
SK (32)	21.8 (7.56 - 36.2)	22 (7.6 - 36.2)	21.8 (7.6 - 36.2)	31.3 (15 - 47)	3.1 (2.1 - 9.1)	9.4 (0.7 - 19.5)	25 (10 - 40)
ON (40)	15 (3.9 - 26)	15 (3.9 - 26)	25 (11.6 - 38.4)	25 (11.6 - 38)	2.5 (2.3 - 7.3)	25 (11.5 - 38.4)	10 (0.7 - 19.3)
ATL (34)	5.8 (2 - 13.8)	23.5 (9 - 37.8)	14.7 (2.8 - 26.6)	23.5 (9 - 37.7)	3 (2.7 - 8.6)	20.6 (7 - 34)	5.8 (2 - 13.8)
ALL (179)	12.8 (8 - 17.8)	18.4 (12.7 - 24)	16.2 (10.8 - 21.6)	20 (14 - 26)	5 (1.8 - 8.2)	15.6 (10.3 - 21)	18 (12.8 - 24)

^a BC = British Columbia, AB = Alberta, SK = Saskatchewan, ON = Ontario,

ATL = Atlantic Canada, ALL = All provinces

^b Addition of new animals 12 months and ^c 5 years before the study.

Table 3: Exposure of beef cow-calf herds to other beef herds in the period between 1997 and 2001 in the study describing biosecurity practices of beef cow-calf herds in

Province ^a / Herds	% herds with feedlot cattle	% herds having fenceline contact with other herds on pasture	% of herds with contact at fairs	% herds lending cow or bulls	% herds borrowing cows or bulls	% herds using communal pasture 2002 - 2003	% herds using communal pasture 1997 - 2001
BC (44)	9.1 (2.5 - 21.7)	68.1 (52.4 - 81.4)	20.4 (9.8 - 35.3)	20.4 (8.5 - 32.4)	13.6 (3.5 - 23.8)	41 (26.8 - 55.4)	41 (26.4 - 55.4)
AB (29)	7 (6.8 - 22.8)	58.6 (39 - 76.5)	20.1 (8 - 39.7)	17.2 (3.4 - 30.9)	10.3 (0.7 - 21.4)	17.2 (3.4 - 31)	24.1 (8.5 - 39.7)
SK (32)	9.4 (2 - 25)	75 (56.6 - 88.5)	28.1 (13.7 - 46.7)	31.2 (15.1 - 47)	12.5 (1 - 24)	40.6 (23.6 - 57.4)	50 (32 - 67.3)
ON (40)	17.5 (7.3 - 32.8)	27.5 (14.6 - 44)	25 (12.7 - 41)	27.5 (13.6 - 41.3)	15 (3.9 - 26.0)	0	0
ATL (34)	8.8 (2 - 23.7)	26.5 (13 - 44.4)	20.6 (8.7 - 38)	32.4 (16.6 - 48)	44.1 (27.4 - 61)	20.6 (7 - 34)	32.4 (16.6 - 48.1)
ALL (179)	10.4 (6.5 - 16.1)	51.2 (42.4 - 57.3)	22.8 (17 - 29.7)	25.7 (19.3 - 32.1)	19.1 (13.2 - 24.7)	24 (17.8 - 30.2)	29 (22.4 - 35.7)

Canada.

^a BC = British Columbia, AB = Alberta, SK = Saskatchewan, ON = Ontario,

ATL = Atlantic Canada, ALL = All provinces

^b A total of 12 out of 43 herds use >1 communal pasture.

Table 4: Exposure of beef cow-calf herds to other species⁵ years prior to the survey (1997 – 2001) in the study describing biosecurity practices of beef cow-calf herds in Canada.

Province / Species	BC (44)	AB (29)	SK (32)	ON (40)	ATL (34)
Pigs	25% (9 – 53.2)	8.3% (1.5 – 35.9)	16.7% (4.7 – 44.8)	16.7% (4.7 – 44.8)	33.3% (13.8 – 61)
Goats	10% (1.8 – 40.4)	10% (1.8 – 40.4)	10% (1.8 – 40.4)	30% (10.8 – 60.3)	40% (16.8 – 68.7)
Sheep	23.5% (9.7 – 47.3)	11.8% (3.3 – 34.3)	17.7% (6.2 – 41)	17.7% (6.2 – 41)	29.4% (13.2 – 53.1)
Chicken / poultry/ litter	22.2% (9 – 45.2)	0	22.2% (9 – 45.2)	6% (30 – 56.4)	22.2% (9 – 45.2)
Bison	25% (4.6 – 70)	0	75% (30.1 – 95.4)	0	0
Llamas / alpacas	0	30% (10.8 – 60.3)	50% (23.7 – 76.3)	10% (1.8 – 40.4)	10% (1.8 – 40.4)
Horses/ other equidae	33.7% (24.6 – 44.2)	15.1% (9.1 – 24.2)	28% (19.5– 38.2)	9.3% (4.8 – 17.3)	14% (8.2 – 22.8)
Captive elk / deer	33.3% (9.7 – 70)	0	50% (18.8 – 81.2)	16.8% (3 – 56.4)	0

^a BC = British Columbia, AB = Alberta, SK = Saskatchewan, ON = Ontario,

ATL = Atlantic Canada, ALL = All provinces

Table 5: The sighting of roaming dogs, foxes and coyotes between 2002 and 2003 in the study describing biosecurity practices of beef cow-calf herds in Canada.

Province ^a / herds	Canids	1 – 10 times/yr	11 – 25 times/yr	>25 times/yr
BC 44	Roaming dogs	21.2 % (13.7 – 31.4)	30.8 % (12.7 – 57.6)	60 % (31.3 – 83.2)
	Foxes	15.5 % (9.6 – 24)	26.3 % (11.8 – 48.8)	16.7 % (3 – 56.4)
	Coyotes / Wolves	8.8 % (4.1 – 17.9)	35.5 % (21.1 – 53.1)	42.6 % (9.7 – 70)
AB 29	Roaming dogs	18.8 % (11.7 – 28.7)	0	10 % (1.8 – 40.4)
	Foxes	14.4 % (8.8 – 22.8)	26.3 % (11.8 – 48.8)	33.3 % (9.7 – 70)
	Coyotes / Wolves	3 % (0.8 – 10.1)	19.6 % (9.2 – 40.7)	32.8 % (22.3 – 45.1)
SK 32	Roaming dogs	10 % (5.2 – 18.5)	7.7 % (1.4 – 33.3)	10 % (1.8 – 40.4)
	Foxes	18.6 % (12.1 – 27.4)	5.3 % (0.9 – 24.6)	33.3 % (9.7 – 70)
	Coyotes / Wolves	19.1 % (11.5 – 30)	22.6 % (11.4 – 39.8)	18 % (10.4 – 29.5)
ON 40	Roaming dogs	30 % (21.1 – 40.7)	30.8 % (12.7 – 57.6)	10 % (1.8 – 40.4)
	Foxes	26.8 % (19 – 36.4)	10.5 % (2.9 – 31.4)	16.7 % (3 – 56.4)
	Coyotes / Wolves	36.8 % (26.3 – 48.6)	9.7 % (3.4 – 25)	1.6 % (0.29 – 8.7)
ATL 34	Roaming dogs	20 % (12.7 – 30.1)	30.8 % (12.7 – 57.6)	10 % (1.8 – 40.4)
	Foxes	24.7 % (17.2 – 34.2)	31.6 % (15.4 – 54)	0
	Coyotes / Wolves	44.2 % (22.4 – 18)	13 % (5.1 – 29)	4.9 % (1.7 – 13.5)
ALL 179	Roaming dogs	45.6 % (38.3 – 52.8)	7.4 % (4.4 – 12.2)	5.7 % (3.1 – 5.7)
	Foxes	54.2 % (46.9 – 61.3)	10.6 % (6.9 – 16)	3.4 % (1.5 – 7.1)
	Coyotes / Wolves	38 % (31.2 – 45.3)	17.3 % (12.5 – 23.5)	34.1 % (27.5 – 41.3)

^a BC = British Columbia, AB = Alberta, SK = Saskatchewan, ON = Ontario,

ATL = Atlantic Canada, ALL = All provinces

Table 6: Use of manure equipment to feed heifers and cows, and spreading manure on pasture land for replacement heifers in the study describing biosecurity practices of beef cow-calf herds in Canada.

Province ^a / Herds	% using manure equipment to feed heifers		% using manure equipment to feed cows		% spreading manure on pasture for replacement heifers
	Occasionally	Regularly	Occasionally	Regularly	
BC (44)	22.7 (11.5 – 37.8)	0.23 (0.0 – 1.2)	25 (13.2 – 40.3)	4.5 (0.1 – 15.5)	13.6 (5.2 – 27.4)
AB (29)	31 (15.3 – 50.8)	0.7 (0.1 – 22.7)	44.8 (26.4 – 64.3)	7 (0.1 – 22.8)	3.4 (0.0 – 17.7)
SK (32)	34.4 (18.6 – 53.2)	0	37.5 (2.1 – 56.3)	0	9.4 (2 – 25)
ON (40)	25 (12.7 – 41.2)	15 (5.7 – 29.8)	25 (12.1 – 41.2)	22.5 (10.8 – 38.5)	35 (20.6 – 51.7)
ATL (34)	11.8 (3.3 – 27.5)	8.8 (2 – 23.7)	23.5 (10.7 – 41.2)	6 (0.1 – 19.7)	41.2 (24.6 – 59.3)
ALL (179)	26.4 (18.5 – 31.6)	4.9 (3.5 – 11.4)	31.2 (23.5 – 37.5)	10 (4.8 – 13.5)	20.5 (15.9 – 28)

^a BC = British Columbia, AB = Alberta, SK = Saskatchewan, ON = Ontario,

ATL = Atlantic Canada, ALL = All provinces

Table 7: Methods employed in manure disposal in the study describing biosecurity practices of beef cow-calf herds in Canada.

Province ^a / Herds	% Injection	% Spread with surface incorporation	% Spread without surface incorporation	Both methods ^b
BC (44)	18.2 (8.2 – 32.7)	34.1 (20.5 – 50)	22.7 (11.5 – 37.8)	25 (13.2 – 40.3)
AB (29)	10.3 (2.2 – 27.4)	38 (20.7 – 57.7)	31 (15.3 – 50.8)	20.7 (8 – 39.7)
SK (32)	9.4 (2 – 25)	18.8 (7.2 – 36.4)	53.1 (34.7 – 71)	12.5 (3.5 – 29)
ON (40)	5 (0.1 – 16.9)	27.5 (14.6 – 44)	37.5 (22.7 – 54.1)	30 (16.6 – 46.5)
ATL (34)	6 (0.1 – 16.9)	14.7 (5 – 31)	23.5 (10.7 – 41.1)	56 (38 – 72.8)
ALL (179)	9.8 (6.1 – 15.4)	26.6 (21 – 34.5)	33.5 (26.1 – 40.4)	28.8 (23 – 37)

^a BC = British Columbia, AB = Alberta, SK = Saskatchewan, ON = Ontario,

ATL = Atlantic Canada, ALL = All provinces

^{b,c}Both methods (Spread with and without surface incorporation)

Table 8: Delivery of feedstuffs on the ground and the access of canids^a to stored grain in the study describing biosecurity practices of beef cow-calf herds in Canada.

Province ^b / Herds	% Hay delivered on the ground	% Green feed / baled cereal delivered on the ground	% Straw delivered on the ground	% Grain delivered on the ground	% Canids having access to stored grain
BC (44)	36.7 (27.5 – 47)	29 (19.6 – 40.6)	21 (13.5 – 31.1)	17.2 (7.6 – 34.6)	20.6 (10.4 – 36.8)
AB (29)	20 (13 – 29.4)	27.5 (1.8 – 39.1)	15.3 (8.2 – 26.5)	24.1 (12.2 – 42.1)	8.8 (3 – 23)
SK (32)	29 (20.5 – 39)	34.8 (24.6 – 46.6)	24.7 (16.6 – 35.1)	38 (22.7 – 56)	11.8 (4.7 – 26.6)
ON (40)	6.7 (3.1 – 13.8)	5.8 (2.3 – 14)	23.5 (15.6 – 33.8)	6.9 (1.9 – 22)	44.1 (29 – 60.1)
ATL (34)	7.8 (3.8 – 15.2)	2.9 (0.8 – 10)	13.6 (7.7 – 22.7)	13.8 (5.5 – 30.6)	14.7 (6.5 – 30.1)
ALL(179)	20 (16.4 – 30.3)	20 (16.4 – 30.3)	19.6 (13.4 – 25.6)	20 (16.4 – 30.3)	20 (16.4 – 30.3)

^a Canids are dogs, cats or wildlife.

^b BC = British Columbia, AB = Alberta, SK = Saskatchewan, ON = Ontario,

ATL = Atlantic Canada, ALL = All provinces

Table 9: Testing for *Trichomonas*, veterinary visits and changing of boots, changing of clothes and washing of boots by visitors / outside employees in the study describing biosecurity practices of beef cow-calf herds in Canada.

Province ^a / Herds	% <i>Trichomonas</i> testing	% Veterinary visits for treatment	% Visitors changing boots	% Visitors washing boots	% Visitors changing clothes
BC (44)	6.8 (0.63 – 14.27)	36.4 (22.2 – 50.6)	13.6 (3.5 – 23.8)	9.1 (0.6 – 17.6)	0
AB (29)	13.8 (1.24 – 56.3)	62.1 (44.4 – 79.7)	10.3 (0.7 – 21.4)	20.7 (6 – 35.4)	0
SK (32)	25 (10 – 40)	68.8 (52.7 – 84.8)	0	12.5 (1.1 – 24)	0
ON (40)	2.5 (4.84 – 7.3)	15 (3.9 – 26.1)	15 (3.9 – 26.1)	30 (15.8 – 44.2)	0
ATL (34)	2.9 (2.74 – 8.62)	5.9 (2.0 – 13.8)	2.9 (2.74 – 8.62)	17.7 (4.8 – 30.5)	2.94 (2.7 – 8.6)
ALL(179)	10.2 (5.2 – 13.8)	37.6 (28.7 – 42.8)	10.5 (4.8 – 13.1)	17.9 (12.3 – 23.5)	2.94 (2.7 – 8.6)

^aBC = British Columbia, AB = Alberta, SK = Saskatchewan, ON = Ontario,

ATL = Atlantic Canada, ALL = All provinces

Table 10: Changing boots, changing clothes and washing boots by the operator when visiting other farms in the study describing biosecurity practices of beef cow-calf herds in Canada.

Province ^a	% Operator spending time on other beef farm	% Operator changing boots	% Operator changing clothes	% Operator washing boots
BC	34.1 (20.1 – 48.1)	11.36 (2 – 20.7)	9.1 (0.6 – 17.6)	11.4 (0.6 – 17.6)
AB	48.2 (30.1 – 66.5)	10.3 (0.74 – 21.4)	6.9 (0.2 – 16.1)	6.9 (0.23 – 16.1)
SK	37.5 (20.7 – 54.3)	6.25 (2.14 – 14.6)	6.3 (0.2 – 14.6)	6.3 (0.2 – 14.6)
ON	50 (34.5 – 65.5)	12.5 (2.25 – 22.8)	15 (3.9 – 26.1)	15 (3.9 – 26.1)
ATL	44.1 (27.4 – 60.8)	11.8 (0.9 – 22.6)	5.9 (0.1 – 13.8)	5.9 (0.1 – 13.8)
ALL	42.7 (35.2 – 49.7)	10.4 (3.1 – 15.1)	8.6 (4.8 – 13.1)	9.1 (7.3 – 22.2)

^a BC = British Columbia, AB = Alberta, SK = Saskatchewan, ON = Ontario,

ATL = Atlantic Canada, ALL = All provinces

Table 11: Bovine Viral Diarrhea virus vaccination for breeding cows, replacement heifers, feeder heifers and steers, and calves, the use of bovine viral diarrhea modified live and killed vaccine for replacement heifers and breeding cows in the study describing biosecurity practices of beef cow-calf herds in Canada.

Province ^a	% BVD breeding cows	% BVD replacement heifers	% BVD heifers and steers	% BVD calves	Breeding Cows		Replacement heifers	
					Modified live vaccine	Killed vaccine	Modified live vaccine	Killed vaccine
BC	28.3 (10 - 33.3)	24 (16.4 - 33.6)	26.2 (18.7 - 35.2)	31.8 (18.6 - 47.6)	25 (13.3 - 42.1)	75 (58 - 86.8)	50 (32.6 - 67.4)	50 (32.6 - 67.4)
AB	20.4 (14 - 28.7)	15.2 (9.3 - 24)	18.7 (12.4 - 27.1)	24.1 (10.3 - 43.5)	47.8 (29.2 - 67.1)	56.5 (36.8 - 74.5)	60 (38.7 - 78.1)	40 (22 - 63.6)
SK	9 (4.9 - 15.5)	7.6 (3.7 - 15)	11.2 (6.2 - 18.6)	15.6 (5.3 - 32.8)	60 (31.3 - 83.2)	40 (16.8 - 68.7)	66.7 (39.1 - 86.2)	33.3 (13.8 - 61)
ON	26.6 (19.3 - 35.4)	34.8 (25.8 - 45)	26.2 (18.7 - 35.2)	20 (9.1 - 35.6)	33.3 (19.2 - 51.2)	66.7 (48.8 - 80.8)	39.3 (23.6 - 57.6)	60.7 (42.4 - 76.3)
ATL	16 (10.3 - 23.8)	18.5 (12 - 27.6)	17.7 (11.7 - 26.1)	5.8 (0.7 - 19.6)	33.3 (16.3 - 56.3)	66.7 (43.8 - 87.2)	42 (23.1 - 63.7)	58 (36.3 - 77)
ALL	20 (16.4 - 30.3)	20 (16.4 - 30.3)	20 (16.4 - 30.3)	23.5 (14.5 - 26.7)	40 (27.7 - 45.1)	53.7 (46 - 62.3)	51.6 (40.2 - 59)	48.4 (41.2 - 60)

5. Conclusions

This objective of this study was to quantify the seroprevalence of *Neospora caninum*, Bovine Leukemia Virus and identify the risk factors contributing to seroprevalence, and to evaluate biosecurity practices in beef cow-calf herds.

In the bovine, the economic importance of *Neospora caninum* lies in the resulting abortions and loss of reproduction in beef cow-calf herds, while the economic importance of Bovine Leukemia Virus infection is certainly less obvious and more related to trade issues.

In the neospora study, “pre-calving use of dry lots”, “separation of cow-calf pair from other cows after calving”, “use of standing water in summer”, “use of running water in winter”, “feeding heifers with manure handling equipment”, “abortion and stillbirths left for canids” and “sightings of wild canids per year” (categorized into three categories: less than 10 times per year, 11– 25 times per year, and greater than 26 times per year) were positively associated with herd serological status.

However, “washing boots between visits to livestock farms” was negatively associated with serological status. Of these 8 variables, only four remained in the final model as risk factors, the use of natural standing water in summer, abortion and stillbirths left for canids, and sightings of wild canids.

We found a significant relationship between herd seropositivity to *N. caninum* and farmers leaving abortions and stillbirths for dogs and wild canids. This association would support the idea that preventing the access of wild canids to fetuses and stillbirths may help to reduce pasture contamination and therefore lower seropositivity in the herd.

Control options are limited; preventing oral exposure to *Neospora* oocysts remains the most viable in preventing neospora infections. Washing of boots was negatively associated with seropositivity to *N. caninum* and this association could prevent transmission of disease.

The low prevalence of Bovine Leukemia Virus (BLV) infection in this study prevented associating risk factors with seroprevalence. However, failure to adhere to good hygiene practices by disinfecting equipment used for castration and dehorning, changing needles between animals during injections and changing sleeves during rectal palpation may increase the likelihood of introducing BLV by cross-contamination thereby increasing morbidity and prevalence of BLV in beef cow-calf herds in the future.

Sourcing of new animals, including calves, from auction pens without pre-purchase screening has the potential for introducing pathogens. The addition of Holstein nurse cows and Holstein calves, considerably high proportion of herds utilizing communal grazing where mating occurred with bulls from other herds

constitutes risky biosecurity practices. It is notable that a small proportion of herds performed breeding soundness and trichomonas testing in bulls.

Cow-calf herds had fence line or “nose-to-nose” contact with cattle from other herds on pasture and attended shows and fairs. Cow-calf herds lend and borrow cows and bulls that returned to herd of origin. Wild canids had access to cow-calf herds as measured by a proxy of number of sightings of roaming dogs, coyotes and foxes and canids.

A high proportion of herds used manure-handling equipment to feed cows and heifers, and high proportion of herds spread manure on pasture for replacement heifers. Cow-calf herds had a high proportion of herds that used the same area for calving and winter-feeding, used hospital pens as maternity pens during calving season and did not separate cow-calf pairs from other cows after calving. A high proportion of herds transported sick animals to veterinary clinic for treatment; a high proportion of herds had cows sharing the same maternity pen, and used dry lot as pre-calving area.

Cow-calf herds had high proportion of herds that used common needle for injecting drugs, used multidose syringes for vaccination and used castration and dehorning equipment without disinfection. A high proportion of herds used common sleeve for rectal palpation and visitors and outside workers did wash or change boots. Cow-calf herds had high proportion of herds that did not vaccinate

breeding cows with BVD virus vaccine in a timely manner, and vaccinated low proportion of cows, heifers and calves.

Controlling for a particular practice may serve as a protective factor, for example, washing of boot when visiting livestock farms was protective for *N. caninum* and perhaps may serve as a protective factor for other infections. Further research is necessary to establish cause and effect relationship in biosecurity practices in beef cow-calf in Canada.

Numerous lapses in management practices observed in this study presents biosecurity concerns and have the potential for allowing pathogen entry into the cowherd. Eliminating sources of infection may be challenging in a beef cow-calf herd, however, controlling for management practices that may introduce infection may be of advantage to overall health and productivity of the herd.